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ENDOCRINOLOGY

VOLUME 35 JULY, 1944 NUMBER 1

INFLUENCE OF HORMONES ON LYMPHOID TISSUE STRUCTURE AND FUNCTION. THE ROLE OF THE PITUITARY ADRENOTROPHIC HORMONE IN THE REGULATION OF THE LYMPHOCYTES AND OTHER CELLULAR ELEMENTS OF THE BLOOD[1]

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NEW HAVEN, CONNECTICUT

INTRODUCTION

THE WIDE VARIATIONS in the numbers of the blood cells in health and disease have not encouraged investigators to postulate a common regulatory mechanism controlling these elements. Yet the existence of numerical limits between which the blood cells fluctuate in the normal subject is remarkable in view of the proliferative capacity of the blood-forming tissues. It seems likely that mechanisms exist which restrain the degree to which the cellular products of these tissues accumulate. An influence of various endocrine glands on hematopoietic tissues has been suggested by several investigators on the basis of clinical observations. This subject has been reviewed by Drinker and Yoffey (1941).

It is the object of this paper to present experimental evidence that the pituitary adrenotrophic hormone is a factor in the regulation of the number of blood lymphocytes. This hormone may also be concerned with the regulation of certain bone marrow elements.

Received for publication April 22, 1944.

[1] A preliminary note has been published (Dougherty, T. F., and A. White. *Science*, 98: 367. 1943).

This investigation has been aided by grants from the Josiah Macy, Jr., Foundation, the International Cancer Research Foundation, and the Fluid Research Fund of Yale University School of Medicine.

[2] Fellow of the International Cancer Research Foundation.

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EXPERIMENTAL

Mice and rats of both sexes were used in these studies. The mice were all of the Strong CBA strain and the rats of the Sprague-Dawley strain. The mice and rats were fed Purina Fox Chow. Adult rabbits and humans were also studied.

In so far as possible, the following conditions were standardized: diet, age of experimental animals, environmental temperature and experimental procedures. Most of the data have been obtained on mice 60 to 80 days old of a single strain (CBA Strong) because of their genetic uniformity and their availability in large numbers. However, supplementary observations have been made with rats (Sprague-Dawley) weighing 200 to 250 gms. and also with rabbits (weight approximately 2.5 kg.) of mixed parentage, and with adult humans. Food and water were available to the animals at all times during the studies. The human subjects were hospital patients on routine hospital diets.

Blood was taken from the tail vein of mice and rats for all determinations. Ear vein blood of rabbits and finger tip blood of humans were used. Total leucocyte and erythrocyte counts were done in the usual manner. Differential leucocyte counts were obtained by counting at least two hundred cells on blood smears stained with May-Grünwald Giemsa. Hemoglobin values were obtained by the alkaline hematin method, using the Evelyn photoelectric colorimeter (Evelyn, 1936). The specific gravity of whole blood was determined by the falling drop method (Kagan, 1941).

The adrenal glands were studied histologically for their lipid content and analyzed quantitatively for total cholesterol. Lipids were stained according to the Herzheimer technic using Sudan IV. Paired mouse adrenals were analyzed for total cholesterol by the method of Schoenheimer and Sperry as modified by Sperry (1938).

All materials were injected subcutaneously in aqueous solution, except where otherwise indicated. Descriptive information regarding the substances administered will be found in the section containing the data.

RESULTS

No effort was made to standardize the relationship between the amount of hormone administered and the body weight of the experimental species, owing to the quantity of hormone which would be necessary for the larger animals. Moreover, it was early observed that relatively small doses of hormone were effective even in the larger species.

Early in these investigations it was apparent that the secretion of the adrenal cortex has a profound influence on the level of certain blood cellular elements. Inasmuch as a variety of unrelated stimuli are known to affect adrenal cortical activity it was necessary to establish in a large number of normal animals the limits of variation of the numbers of blood cells under laboratory conditions. A possible diurnal variation in the blood picture has been checked by taking blood from different animals at various times during the day. Data on control animals are collected in table 1 and serve for comparison with values

obtained in the various experiments. Experimental data for particular blood constituents are considered significant when the values exceed the limits of the average deviation obtained in the control animals.

It should be noted that although the segmented forms are referred to in the tables as polymorphonuclear cells, this is done as a matter of convenience in tabulating data for various species. These cells in the rabbit are pseudoeosinophils and in the rat and the mouse

TABLE 1. AVERAGE VALUES AND THEIR DEVIATION, FOR BLOOD CONSTITUENTS OF THE CONTROL MICE, RATS AND RABBITS

Species	No. of animals	Hemoglobin	R.B.C.	W.B.C.	Lymphocytes	Polymorphonuclear leucocytes	Specific gravity whole blood
		gm./%	millions/cu. mm.				
Mouse	99	15.3±1.06[1]	9.05±0.85[1]	12,814±3,632	9,584±3,047	3,257±1,304	1.060±0.0025[2]
One day adrenalectomised mouse	60	15.4±1.10[4]	8.65±1.39[4]	14,903±3,511	11,717±2,826	3,189±1,247	1.061±0.0016[9]
Rat	42	14.8±0.61[5]	8.88±0.91[5]	17,826±4,545	14,700±4,027	2,932±1,243	1.059±0.0008[8]
Rabbit	18	12.0±1.38[7]	5.67±0.77[7]	10,053±1,730	6,861±1,723	3,193±1,682	1.053±0.0009[9]

[1] 39 animals. [2] 19 animals. [3] 5 animals. [4] 12 animals. [5] 27 animals. [6] 8 animals. [7] 16 animals. [8] 3 animals.

should probably be referred to as heterophils. Eosinophils are not grouped separately since they are few in number and apparently were unaffected by the experimental procedures.

Even under conditions standardized as carefully as possible, the alterations in blood picture produced by administration of the various agents used will depend upon (1) the normal blood picture of the species studied, (2) the quantity of agent administered, and (3) the route of administration.

Effect of Adrenotrophic Hormone Injection

Blood analyses were conducted at intervals after hormone injection, using different groups of mice at each time interval, rather than making successive determinations on the same animals.[3] Striking alterations occur in the numbers of blood cells of mice within the first few hours following a single subcutaneous injection of 1.0 mg. of purified pituitary adrenotrophic hormone (Sayers, White and Long, 1943) dissolved in 0.5 ml. of water. The blood picture obtained is characterized by a decrease in total leucocyte count, a decrease in absolute number of lymphocytes, and an increase in absolute number of polymorphonuclear cells. The alterations in the numbers of leucocytes are evident at one hour following hormone injection. The maximum lymphopenic effect is observed at 9 hours after injection. Shortly

[3] Initially in these studies it was considered inadvisable to conduct successive bleedings on the same mouse because of the possible influence of blood loss on the blood picture. However, 10 mice bled successively at 3 hour intervals over a 9 hour period showed no significant alterations from the initial blood picture. Similar results have been obtained with adrenalectomized mice. When larger animal species were studied the influence of bleeding was not considered.

thereafter the leucocyte picture tends to return to normal. These changes are depicted in figure 1.

The pattern of the changes illustrated in figure 1 is not invariably the same. The species of animal, and indeed the dose of hormone and method of administration within the same species, may affect the leucocyte picture. The degree and type of leucocyte changes and the time at which they are evident must depend on the rate at which the released adrenal cortical secretion reaches the lymphocytic elements.

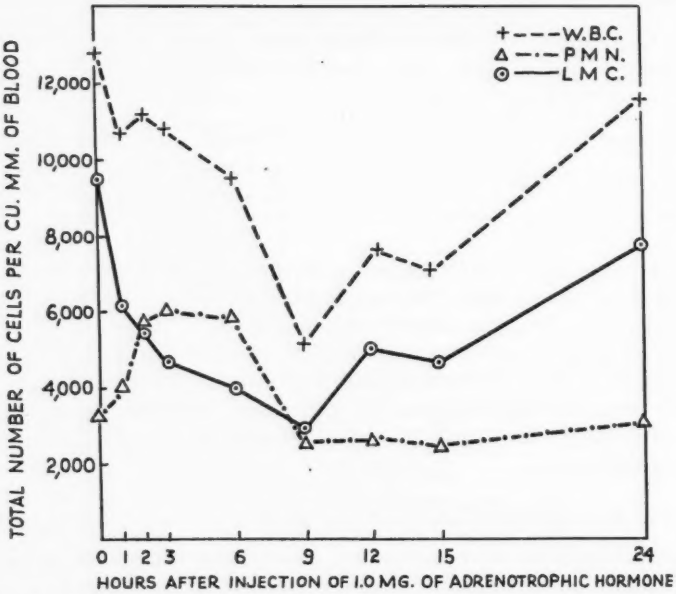

FIG. 1. Alterations produced in the total leucocyte, lymphocyte and polymorphonuclear blood counts of normal mice receiving, at zero time, a single, subcutaneous injection of 1.0 mg. of pituitary adrenotrophic hormone. The points on each curve at 0, 1, 2, 3, 6, 9, 12, 15 and 24 hours are, respectively, the averages of data for groups of 99, 3, 3, 5, 5, 7, 6, 7, and 7 animals.

Some of the species variations will be mentioned for each agent administered.

An initial increase in hemoglobin and red blood cells was generally observed within 3 to 6 hours after a single injection of adrenotrophic hormone. Subsequently, the hemoglobin concentration and the red blood cells fell to subnormal levels which persisted as long as 24 hours after the injection. The changes were not accompanied by significant alterations in the specific gravity of the whole blood. These data are shown in figure 2.

A marked decrease in total adrenal cholesterol occurs 3 hours after intraperitoneal injection of adrenotrophic hormone in the 21

day-old rat (Sayers, Sayers, White and Long, 1943; Sayers, Sayers, Fry, White and Long, 1944). This has been interpreted to be a criterion of adrenal cortical secretion. Since the most marked lymphopenia was observed in mice at approximately 9 hours following subcutaneous injection of adrenotrophic hormone, it was of interest to determine whether a correlation existed between the degree of lymphopenia and the extent of depletion of adrenal cholesterol and lipids.

For this study, mice were given single subcutaneous injections of 1.0 mg. of adrenotrophic hormone (in 0.5 ml. water) and sacrificed at 1, 2, 3, 6, 9 and 24 hours after injection. Groups of 3 animals were sacrificed at each interval. Total cholesterol analyses were conducted

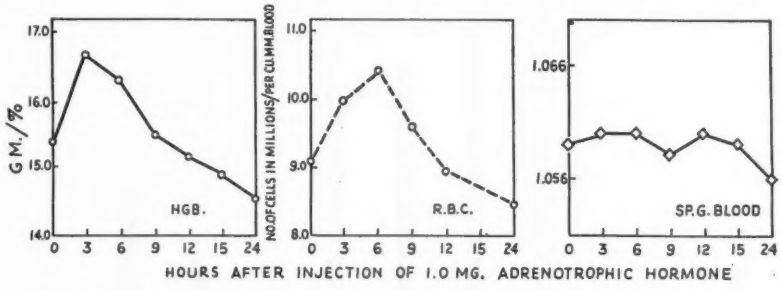

FIG. 2. Alterations produced in the total hemoglobin, total red cell count, and whole blood specific gravity of normal mice receiving, at zero time, a single, subcutaneous injection of 1.0 mg. pituitary adrenotrophic hormone. Each point on the curves is the average of data obtained for at least 5 animals.

on both adrenals from each of 2 animals; the adrenals of the third animal were fixed for Sudan staining. The cholesterol changes are shown in figure 3. As in the 21 day-old rat, adrenotrophic hormone injection in the mouse produces a distinct lowering of the level of total cholesterol in the adrenals. Depletion of adrenal sudanophilic material parallels cholesterol loss. Most striking is the similarity in the downward trends of both the adrenal cholesterol levels in figure 3 and the lymphocyte counts in figure 1.

In the rat, subcutaneous injection of 2.0 mg. of adrenotrophic hormone produces blood changes similar to those illustrated in figures 1 and 2 for mice. The level of blood lymphocytes was maintained at a lower value for a longer period of time following adrenotrophic hormone injection in the rat as compared to the mouse. Like mice, rats exhibited a definite polymorphonuclear leucocytosis. The hemoglobin concentration and red cell counts in rats given adrenotrophic hormone are also similar in trend to those observed in the mouse. The data are presented in table 2.

Rabbits show marked blood changes after the subcutaneous injection of 10 mg. of hormone. The maximum effect on the blood leucocytes occurs within 3 hours (table 2). The alterations in lymphocytes

and polymorphonuclear cells are still evident in the rabbit 24 hours after hormone injection. Rats and rabbits, although receiving less hormone per unit of body weight, showed more profound and lasting effects than did the mouse.

Having established the effects of adrenotrophic hormone injection on the blood picture, the question arose as to whether all of the effect of this hormone was mediated through the adrenal cortex or whether a direct peripheral mechanism was also involved. The subcutaneous

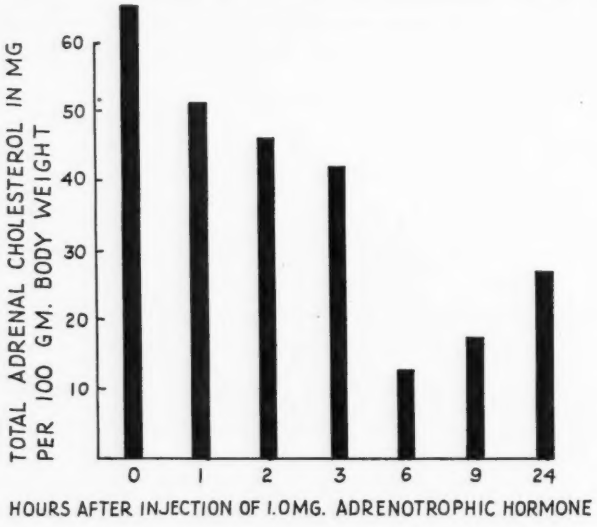

FIG. 3. Effect of a single injection of adrenotrophic hormone on the total adrenal cholesterol of normal mice.

injection of adrenotrophic hormone into mice one day after adrenalectomy does not produce leucopenia or lymphopenia. However, in the adrenalectomized animal adrenotrophic hormone produces an increase in the polymorphonuclear cells similar to that seen in the injected intact mouse.

The role of the pituitary adrenotrophic hormone in the control of blood lymphocyte levels is demonstrated further by the fact that the injection of 5.0 mgs. of this hormone into 2 hypophysectomized rats produced a decrease of approximately 5000 lymphocytes in each animal within 9 hours. This effect is similar to that seen in intact animals injected with adrenotrophic hormone and in adrenalectomized mice and rats given adrenal cortical extract.

Further evidence that adrenotrophic hormone affects blood constituents because of its hormonal action and not because of its protein nature is seen from the fact that 8 normal mice injected subcutane-

ously with 2.0 mg. of purified serum gamma globulin (human)[4] showed no lymphopenia when examined 3, 6 and 9 hours after injection. Also, 7 adrenalectomized mice injected with adrenotrophic hormone did not exhibit the lymphopenic effect. All of the evidence suggests that the blood changes reported are not due to the protein nature of the hormone.

TABLE 2. AVERAGE CHANGES IN BLOOD CONSTITUENTS RESULTING FROM A SINGLE, SUBCUTANEOUS INJECTION OF 5.0 MG. OF ADRENOTROPHIC HORMONE IN RATS AND 10.0 MG. OF HORMONE IN RABBITS

Number of animals	Time after injection	Hemoglobin	R.B.C.	W.B.C.	Lymphocytes	Polymorphonuclear leucocytes	Specific gravity whole blood
Rats	hours	gm./%	millions/ cu. mm.				
42	0	14.8[1]	8.88[1]	17,826	14,700	2,932	1.059[2]
12	3	15.3	9.48	7,634	4,016	3,240	1.058
12	6	15.1	8.43	9,712	3,873	5,884	1.059
8	9	14.6	8.76	10,551	3,386	7,111	1.061
12	24	13.0	7.36	11,819	7,782	3,776	1.055
Rabbits							
18	0	12.0[3]	5.67[3]	10,053	6,861	3,193	1.053[4]
4	3	11.5	5.69	7,175	2,562	4,597	1.054
4	6	11.3	5.94	10,338	2,196	8,149	1.061
4	9	11.2	5.26	11,925	3,877	8,051	1.057
2	12	11.2	7.42	13,775	3,724	10,051	1.055
3	24	—	—	11,961	5,066	6,895	—

[1] 27 animals. [2] 19 animals. [3] 16 animals. [4] 3 animals.

Effect of Injection of Adrenal Cortical Hormone

Aqueous adrenal cortical extract (Wilson) was used in most of the experiments presented in this section. The subcutaneous injection of 0.5 ml. of this extract in mice produces an extreme lymphopenia within 3 hours (table 3). As in mice given adrenotrophic hormone, there is an accompanying polymorphonuclear leucocytosis. In animals receiving adrenal cortical extract, the lymphocytes return to normal levels within 6 hours after injection; elevated polymorphonuclear counts may persist at this time. The maximum blood alterations in mice given adrenal cortical extract occur earlier than in animals treated with adrenotrophic hormone. It should again be emphasized that these relationships are described for a particular dose of hormone administered subcutaneously in the mouse.

In adrenalectomized mice, subcutaneous injection of 0.5 ml. of adrenal cortical extract produces blood alterations which are indistinguishable from those seen in unoperated animals. The comparison is plotted in figure 4. The number of lymphocytes in animals one day after adrenalectomy is higher than in intact animals.

Intact mice given adrenal cortical extract showed decreases in

[4] Kindly prepared by Dr. L. S. Ciereszko of Sharp & Dohme, Inc.

hemoglobin and red cell counts similar to the animals receiving adrenotrophic hormone. However, with cortical extract no initial hemoglobin and red cell increases were observed (table 3). The data presented in this table also show that injection of aqueous adrenal cortical extract produced a lymphopenia in rats and in rabbits. The response to the hormone is similar in these species. It is interesting that adrenotrophic hormone has a more profound lymphopenic effect in the rabbit

TABLE 3. AVERAGE CHANGES IN BLOOD CONSTITUENTS RESULTING FROM A SINGLE, SUBCUTANEOUS INJECTION OF 0.5 ML. AQUEOUS CORTICAL EXTRACT IN MICE, 2.0 ML. IN RATS, AND 5.0 ML. IN RABBITS

Number of animals	Time after injection	Hemoglobin	R.B.C.	W.B.C.	Lymphocytes	Polymorphonuclear leucocytes	Specific gravity whole blood
	hours	gm./%	millions/ cu. mm.				
				Mice			
99	0	15.3[1]	9.05[1]	12,814	9,584	3,257	1.060[2]
8	3	13.8	7.92	8,019	3,453	4,452	1.061
8	6	13.6	9.07	12,436	7,888	4,119	1.062
8	9	13.0	7.18	11,500	7,414	5,086	—
				Rats			
30	0	14.8[3]	8.88[3]	17,826	14,700	2,932	1.058[4]
4	3	15.3	9.52	6,203	3,574	2,629	1.058
4	6	14.3	8.88	9,340	3,999	5,341	1.057
4	24	13.6	8.07	9,588	6,341	3,247	1.056
				Rabbits			
18	0	12.0[5]	5.67[5]	10,053	6,861	3,193	—
2	1	—	—	8,650	4,315	4,335	—
4	3	11.3	5.93	10,650	4,715	5,935	—
4	6	11.8	6.38	14,382	5,506	8,857	—
4	9	11.9	5.64	15,740	9,682	6,043	—
4	24	11.3	5.30	10,462	7,189	3,278	—

[1] Average for 39 animals.
[2] Average for 19 animals.
[3] Average for 27 animals.
[4] Average for 8 animals.
[5] Average for 16 animals.

as compared to the response of this species to adrenal cortical extract. In the mouse, on the other hand, this difference in behavior to the types of hormone preparations was not seen.

Table 4 presents data obtained following subcutaneous injection of aqueous adrenal cortical extract in human beings.[5] The tendency toward a decrease in red cells, lymphocytes and hemoglobin resembles the alterations in blood picture seen in other species treated with hormone. With the dose of hormone employed, the blood changes in man were not as consistent, although the fall in total lymphocytes was relatively even more profound because of the lower initial lymphocyte level. A lymphopenia was not evident in all patients studied following every injection of hormone but was usually observed

[5] Acknowledgment is made to Dr. E. A. Lawrence and Dr. P. H. Lavietes for coöperating in the study of patients.

(unpublished results). Whether this less consistent response in man is a dose factor or individual variation cannot be decided at this time, since the data are too few. However, it is recognized that the dose of cortical extract used in the human studies is relatively small when compared on a body weight basis to doses used in the laboratory animals.

The effects of several adrenal cortical steroids on the blood picture of the mouse are presented in table 5. The injection of desoxycorti-

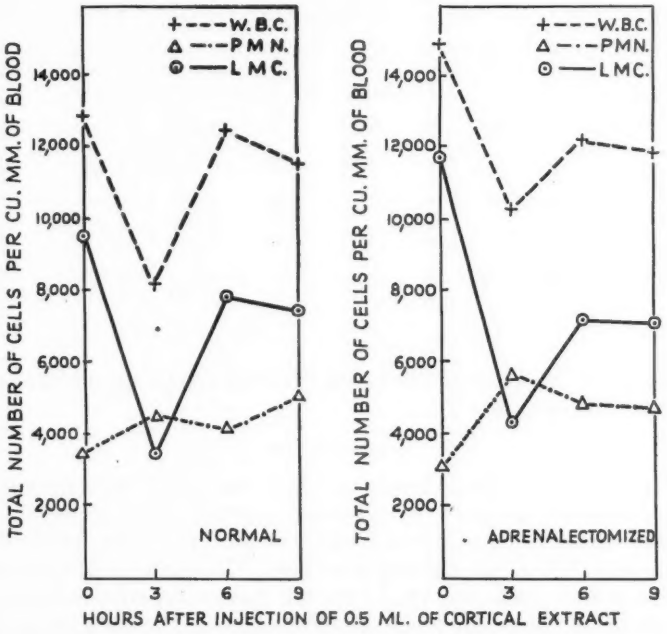

FIG. 4. Effect of subcutaneous injection of 0.5 ml. aqueous adrenal cortical extract on the white blood cell counts of normal and adrenalectomized mice. Each experimental point on the curves for normal animals represents the average of data obtained for a group of 8 mice. Groups of 6 animals were used to establish the experimental points on the curves for adrenalectomized mice.

costerone acetate (in sesame oil, Schering), even in relatively large doses produces no significant decrease in lymphocytes in normal or in adrenalectomized mice. An increase in polymorphonuclear leucocytes occurs after injections of desoxycorticosterone acetate. In contrast to the inability of this steroid to produce a lymphopenia, cortical steroids in oil (Upjohn),[6] corticosterone, or the adrenal cortical steroid called

[6] Grateful acknowledgment is made to Dr. E. Gifford Upjohn of The Upjohn Company for supplying this preparation.

TABLE 4. BLOOD CELL ALTERATIONS IN PATIENTS FOLLOWING SINGLE, SUBCUTANEOUS INJECTIONS OF AQUEOUS ADRENAL CORTICAL EXTRACT

Patient	Diagnosis	Amt. hormone injected	Time after injection	Hemoglobin	R.B.C.	W.B.C.	Lymphocytes	Polymorphonuclear leucocytes
		ml.	hours	gm./%	millions/ cu. mm.			
D.	lymphosarcoma	20	0	9.4	2.85	7,750	1,550	6,200
			3	8.6	3.25	8,300	587	7,713
			6	8.53	3.07	8,850	1,356	7,045
D.	lymphosarcoma	20	0	9.9	2.91	9,210	2,310	6,900
			3	8.6	2.51	7,050	987	6,063
			6	8.0	3.34	6,850	1,300	5,550
M.	lymphosarcoma	30	0	13.2	5.19	9,600	3,840	5,760
			3	14.0	3.83	7,000	2,032	4,970
			6	13.0	4.36	8,000	3,360	4,640
M.	lymphosarcoma	30	0	13.7	5.20	7,800	2,964	4,836
			3	13.7	3.95	5,300	2,014	3,286
			6	13.0	5.34	7,900	2,370	5,530
Q.	myxedema-lymphosarcoma	25	0	—	—	6,300	3,275	3,025
			3	—	—	4,500	1,710	2,795

compound F by Wintersteiner,[7] all produce lymphopenia in adrenalectomized mice (table 5).

DISCUSSION

The numerical limits between which the blood cells fluctuate in normal animals suggests the existence of regulatory processes. These mechanisms may be concerned with (a) the production of the blood cells, and (b) the disposition of these cells. The dynamic balance which obtains at any particular time between these mechanisms determines the nature of the cellular picture of the blood. The data presented in this paper establish the pituitary adrenotrophic hormone as a prime factor in the regulation of the level of blood lymphocytes. This regulatory influence is exerted by the trophic action of this hormone on the adrenal cortex. Furthermore, the adrenotrophic hormone may also influence the number of circulating red cells.

The conclusion that the pituitary adrenotrophic hormone is concerned with blood lymphocyte levels is based upon the finding that the injection of this hormone or the products of its stimulatory action, i.e., adrenal cortical substances, into a variety of normal animal species produces a profound, absolute lymphopenia. The same result is ob-

[7] Compound F of Wintersteiner is the same substance as compound E of Kendall and compound Fa of Reichstein (Reichstein and Shoppee, 1943). Grateful acknowledgement is made for gifts to Dr. C. N. H. Long by Dr. Wintersteiner of the sample of compound F and by Dr. Kendall of the corticosterone.

TABLE 5. AVERAGE ALTERATIONS IN BLOOD CONSTITUENTS FOLLOWING SINGLE, SUBCUTANEOUS INJECTIONS OF ADRENAL CORTICAL STEROIDS IN NORMAL AND IN ADRENALECTOMIZED MICE

Preparation and animal used	No. of animals	Dose	Time after injection	W.B.C.	Lymphocytes	Polymorphonuclear leucocytes
		mg.	hours			
Desoxycorticosterone acetate in sesame oil injected into normal mice	99	0.1	0	12,814	9,854	3,257
	5		3	13,680	7,929	5,751
	3		6	16,600	9,339	7,260
	3		9	17,667	9,266	8,400
Desoxycorticosterone acetate in sesame oil injected into normal mice	3	0.25	0	12,814	9,854	3,257
	3		3	26,700	13,269	13,431
	3		6	28,500	13,190	15,310
	3		9	15,050	8,995	6,065
Desoxycorticosterone acetate in sesame oil injected into adrenalectomized mice	60	0.1	0	14,903	11,717	3,189
	3		3	13,833	9,133	4,693
	3		6	10,700	8,640	2,060
	3		9	18,375	14,591	3,784
Desoxycorticosterone acetate in sesame oil injected into adrenalectomized mice	60	2.5	0	14,903	11,717	3,189
	3		3	16,033	10,357	5,676
	3		6	16,100	10,280	5,820
	3		9	13,517	10,277	3,263
Adrenal cortical steroids in oil injected into normal mice	99	0.1	0	12,814	9,854	3,257
	7		3	18,093	3,158	4,935
	3		6	18,933	10,088	8,845
	3		9	16,633	10,680	5,953
Adrenal cortical steroids in oil injected into adrenalectomized mice	60	0.1	0	14,903	11,717	3,189
	9		3	11,617	7,430	4,292
	3		6	13,617	12,157	1,460
	3		9	20,000	17,323	2,677
Compound F (Wintersteiner) injected into aqueous solution into adrenalectomized mice	60	0.25	0	14,903	11,717	3,189
	6		3	24,567	14,009	10,560
	6		6	14,767	6,733	8,034
	6		9	11,040	7,282	3,756
	3		24	14,488	10,030	4,387
Corticosterone injected in aqueous solution into adrenalectomized mice	60	0.25	0	14,903	11,717	3,189
	6		3	20,575	11,037	9,638
	6		6	15,400	8,050	7,356
	6		9	16,000	11,172	4,827
	3		24	16,767	10,560	6,207

served in the hypophysectomized rat given adrenotrophic hormone and in the adrenalectomized mouse treated with adrenal cortical extracts. Evidence that the adrenotrophic hormone is concerned with the disposition of lymphocytes is seen in the lymphocytosis resulting following adrenalectomy (Zwemer and Lyons, 1928; Corey and Britton, 1932), or hypophysectomy (unpublished results). The term "lymphocyte disposition" may have a two-fold interpretation. It may indicate either a peripheral removal of lymphocytes, or a decreased delivery of lymphocytes to the circulation. Either or both of these mechanisms would result in lymphopenia. Evidence available from

histological studies (White and Dougherty, in press) suggests that the adrenotrophic hormone-induced lymphopenia is a result of a failure of delivery of adequate numbers of lymphocytes to the circulation.

The most profound lymphopenia occurs within a few hours following hormone injection. The tendency for the lymphocyte count to return to normal was observed in almost all animals within 24 hours after a single injection, although in the rat low lymphocyte levels were evident at this time. The effect of the hormone is an acute one, and delivery of the lymphocytes to the blood is rapidly restored to normal. Changes in blood lymphocytes are mirrored by the alterations in adrenal cholesterol concentration. Adrenal cholesterol levels have been shown to be lowered by stimulation of the adrenal cortex (Sayers, Sayers, White and Long, 1943; Sayers, Sayers, Fry, White and Long, 1944.

Although adrenotrophic hormone exerts a regulatory effect on the level of blood lymphocytes, it appears that the hormone does not directly influence the number of blood polymorphonuclear cells. A polymorphonuclear leucocytosis results from injection of adrenotrophic hormone into adrenalectomized animals, and therefore cannot be a specific hormonal effect in this case. A possible role of the adrenal cortex in regulation of blood polymorphonuclear cells is suggested by the work of Corey and Britton (1932) who found a markedly decreased number of these cells in adrenalectomized cats.

The injection of adrenotrophic hormone or adrenal cortical extract into normal mice produces an initial, transitory increase in red cells and hemoglobin followed by a decrease. In general, the red cell and hemoglobin values are distinctly below normal 24 hours following a single injection of hormone. The anemia observed cannot be attributed to changes in blood specific gravity.

The results which have been described for adrenotrophic hormone injection are a result of the specific physiological action of this hormone as a humoral agent and are not due to its protein nature. This is evident from the fact that serum gamma globulin injections in normal mice, and adrenotrophic hormone in adrenalectomized mice, did not influence the blood picture.

Injections of desoxycorticosterone acetate into normal or adrenalectomized animals did not reduce blood lymphocytes. In contrast, adrenal cortical compounds, e.g., adrenal cortical steroids in oil, compound F of Wintersteiner (compound E of Kendall), or corticosterone have a lymphopenic activity in adrenalectomized animals. It is significant that adrenal cortical steroids which have a glyconeogenic function (Long, Katzin and Fry, 1940) are also compounds which induce lymphopenia. Desoxycorticosterone, which does not affect carbohydrate formation from protein, does not decrease blood lymphocytes. The basis for the regulatory role of the adrenal cortex in gly-

coneogenesis may lie not primarily in its influence on the intermediary reactions of carbohydrate formation from protein, but in its capacity to supply protein to the blood (White and Dougherty, in press) and thus to the liver, from lymphoid tissue and perhaps other structures. Wells and Kendall (1940) have reported thymic involution with corticosterone and with a highly active concentrate of adrenal cortical steroids, but not with desoxycorticosterone. Data have been obtained (Dougherty and White, 1943) which demonstrate that the involution of thymic mass after adrenotrophic hormone injections is accompanied by an involution of other lymphoid tissue. This suggests that the effects of adrenotrophic hormone on numbers of blood lymphocytes and on lymphoid tissue are directly correlated. Many non-specific toxic agents which have been reported (Selye, 1937) to decrease thymic size would be expected to produce a lymphopenia, and vice versa. It is not unlikely that these non-specific factors cause some of their effects on lymphoid tissue through the pituitary adrenal cortex relationship, although large doses of toxic agents may have a direct action on lymphoid cells.

The fact that a continued lymphopenia may not be evident in conditions of chronic stimulation of the adrenal cortex would indicate that a balance exists between the proliferative capacity of the lymphoid tissue and the rate of removal of lymphocytes. The two processes, i.e., lymphocyte production and lymphocyte disposition, may be completely independent or related indirectly to one another. The evidence in this paper suggests a pituitary effect only on lymphocyte disposition. However, acceleration of this phenomenon might induce an exaggerated rate of production of lymphoid cells. Therefore, the blood lymphocyte picture and the lymphoid tissue structure may vary widely under normal and pathological conditions, depending on the relationship existing at a particular time between lymphoid cell production and disappearance.

The similarity of the lymphocyte pattern resulting from pituitary adrenal cortical stimulation to that seen in acute infectious processes, reactions to toxins (bacterial and otherwise) and to a variety of unrelated stimuli, indicates that the well known effect of these agents on the adrenal cortex (Sayers, Sayers, Fry, White and Long, 1944) may be the fundamental basis of the lymphocyte picture which has been described for these conditions. The data presented here may find application in the evaluation of adrenal cortical activity.

SUMMARY

Single injections of pituitary adrenotrophic hormone in mice, rats and rabbits produce within a few hours an absolute lymphopenia and an increase in polymorphonuclear leucocytes. The lymphopenia is a specific response to the hormone, because (a) it does not occur in adrenalectomized animals treated with adrenotrophic hormone, or

(b) in intact animals treated with a pure protein. The polymorphonuclear response is not specific in that it does occur in adrenalectomized animals given a variety of agents.

Single injections of adrenotrophic hormone or of adrenal cortical extract into intact mice and rats result in slight decreases in red cell and hemoglobin concentration.

Adrenal cortical extract, adrenal cortical steroids in oil, corticosterone, or compound F (Wintersteiner) produce a lymphopenia in intact and adrenalectomized animals. On the other hand, desoxycorticosterone does not decrease the total number of blood lymphocytes in either normal or operated animals.

The regulation of the numbers of blood lymphocytes, and probably the red cells, is under pituitary control and is mediated by way of the adrenal cortex.

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REFERENCES

COREY, E. L., AND S. W. BRITTON: *Am. J. Physiol.* 102: 699. 1932.
DOUGHERTY, T. F., AND A. WHITE: *Proc. Soc. Exper. Biol. & Med.* 53: 132. 1943.
DRINKER, C. K., AND J. M. YOFFEY: Lymphatics, lymph and lymphoid tissue. Harvard Univ. Press. Cambridge. 1941.
EVELYN, K. A.: *J. Biol. Chem.* 115: 63. 1936.
KAGAN, B. M.: *J. Lab. & Clin. Med.* 26: 1681. 1941.
LONG, C. N. H., B. KATZIN AND E. G. FRY: *Endocrinology* 26: 309. 1940.
REICHSTEIN, T., AND C. W. SHOPPEE: Vitamins & Hormones, Vol. 1, p. 346. Academic Press, New York, 1943.
SAYERS, G., M. A. SAYERS, E. G. FRY, A. WHITE AND C. N. H. LONG: *Yale J. Biol. & Med.* 16: 361. 1944.
SAYERS, G., M. A. SAYERS, A. WHITE AND C. N. H. LONG: *Proc. Soc. Exper. Biol. & Med.* 52: 200. 1943.
SAYERS, G., A. WHITE AND C. N. H. LONG: *J. Biol. Chem.* 149: 425. 1943.
SELYE, H.: *Endocrinology* 21: 169. 1937.
SPERRY, W. M.: *Am. J. Clin. Path.* 2: 91. 1938.
WELLS, B. B., AND E. C. KENDALL: *Proc. Staff Meet., Mayo Clin.* 15: 133. 1940.
WHITE, A., AND T. F. DOUGHERTY: *Proc. Soc. Exper. Biol. & Med.* 56: 26. 1944.
ZWEMER, R. L., AND C. LYONS: *Am. J. Physiol.* 86: 545. 1928.

METABOLISM OF THE STEROID HORMONES: THE ADRENAL GLAND AS THE SOURCE OF CORTIN-LIKE MATERIAL IN THE URINE OF MONKEYS[1]

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THAT CORTIN-LIKE MATERIAL is excreted in the urine of normal men and post-operative patients has been previously demonstrated. Extracts of normal men's urine have been shown to possess the following biological activities in the adrenalectomized rat: protection against low environmental temperatures (Dorfman, Horwitt and Fish, 1942), maintenance of life (Dorfman and Horwitt, 1943), increased work performance of muscle (Shipley, Dorfman and Horwitt, 1943), increased formation of liver glycogen (Horwitt and Dorfman, 1943), and protection against water intoxication (Schiller and Dorfman, 1943). Post-operative urine has been shown to possess material capable of protecting adrenalectomized rats against cold (Weil and Browne, 1939) as well as maintaining life and increasing the synthesis of liver glycogen (Venning, Hoffman and Browne, 1943).

This report deals with the presence of cortin-like material in the urine of monkeys. The studies which have been made on intact, gonadectomized, adrenalectomized, and gonadectomized-adrenalectomized monkeys are highly suggestive that the urinary cortin-like material is of adrenal origin.

ANIMALS, MATERIAL, METHODS

Five male and four female rhesus monkeys were used. Four of the animals had been at the laboratory for about four years, and were in good health. The remaining five animals were purchased from animal dealers and were in good health on arrival at the laboratory. None of the nine animals showed signs of disease at autopsy. Prior to adrenalectomy, the monkeys were maintained on Purina Dog Chow supplemented by cabbage and oranges. After adrenalectomy, they usually lost their appetite for the dog chow and then subsisted on oranges, apples, carrots, cabbage, potatoes and eggs. The animals were kept in metabolism cages and the urine was collected daily under toluene.

Adrenalectomy was performed in two stages under ether anaesthesia. The approach was retroperitoneal. After the operation, when not receiving cortical hormones, the animals were maintained by intraperitoneal injections of sodium chloride.

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The fresh urine was extracted three times with one-fourth volume of ethylene dichloride at room temperature. The ethylene dichloride extracts were evaporated to dryness in vacuo, taken up in small volumes of absolute ethanol and again evaporated to dryness. The residue was taken up in 95 per cent ethanol and diluted with water.

Albino rats of the Sprague-Dawley strain were employed for assay. Male rats between 23 and 25 days of age, and weighing between 35 and 50 gr. were bilaterally adrenalectomized under ether anaesthesia. Twelve to 24 hours after operation, the dose of urinary extract contained in 1 cc. of a 10 per cent ethanol solution was administered by stomach tube. Control rats received only the solvent. After the urinary extract or solvent was administered, the rats were put into individual glass cages without food and water and placed in the cold room at 5.5°±1° C. The rats were observed at one-half hour intervals and the time of death was noted. The animals were judged dead when no signs of respiration were observed and when the rats showed no movement after shaking the cage. Control and urinary extract-treated animals were always run simultaneously. Significance of the differences between mean survivals were calculated from Fischer's "t" table.

RESULTS

The urinary excretion of cold-protecting material by intact male monkeys is tabulated in Table 1. Of eleven observations on five adult

TABLE 1. THE EXCRETION OF COLD-PROTECTING MATERIAL BY INTACT ADULT MALE MONKEYS

(Urinary Extract Derived from 20 Hours of Urine Administered to Each Adrenalectomized Rat. Each Group of Rats Consisted of 10 or 11 Animals.)

Monkey		Control rats	Urinary extract treated rats	Increase in mean survival %	P
Number	Weight in kilos	Mean survival hours	Mean survival hours		
1	8.4	7.4±0.28*	10.0±0.98	35	0.02
		6.0±0.56	6.2±0.84	3	>0.05
		4.6±0.42	8.5±0.48	85	0.01
3	7.6	4.9±0.42	6.1±0.52	25	0.05
		5.7±0.44	6.0±0.37	5	>0.05
4	6.9	4.9±0.42	6.5±1.02	33	0.05
		5.7±0.44	6.1±0.58	7	>0.05
5	11.0	4.9±0.40	6.8±0.66	39	0.05
		5.7±0.44	5.5±0.36	−4	>0.05
		5.7±0.44	7.0±0.81	23	>0.05
8	6.0	7.7±0.29	8.7±0.64	13	>0.05

* Standard Error.

male monkeys, five instances of significant differences between the mean survivals of control and urinary extract treated rats were found. Table 2 lists the results on the urinary cortin in female monkeys. Of ten determinations on four animals, six positive results were observed.

Table 3 deals with the presence of cold-protecting material in the urines of intact, ovariectomized, adrenalectomized, ovariectomized

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TABLE 2. THE URINARY EXCRETION OF COLD-PROTECTING MATERIAL BY INTACT ADULT FEMALE MONKEYS

(Urinary Extract Derived From 20 Hours of Urine Administered to Each Adrenalectomized Rat. Each Group of Rats Consisted of 9 to 11 Animals)

Monkey		Control rats	Urinary extract treated rats	Increase in mean survival %	P
Number	Weight in kilos	Mean survival hours	Mean survival hours		
2	5.2	7.4 ± 0.28*	10.2 ± 1.16	38	0.05
		4.8 ± 0.58	10.8 ± 0.67	83	0.01
		4.6 ± 0.45	6.8 ± 0.87	48	0.05
6	4.5	5.7 ± 0.44	6.2 ± 0.55	9	>0.05
		4.8 ± 0.40	6.8 ± 0.82	42	0.05
		5.6 ± 0.27	8.8 ± 0.49	57	0.01
7	6.6	5.7 ± 0.44	5.6 ± 0.31	− 2	—
		5.7 ± 0.49	4.5 ± 0.31	−21	—
		7.7 ± 0.29	6.3 ± 0.28	−18	—
9	4.2	3.1 ± 0.26	3.6 ± 0.23	16	0.02

* Standard Error.

TABLE 3. THE URINARY EXCRETION OF COLD-PROTECTING MATERIAL BY INTACT, OVARIECTOMIZED, ADRENALECTOMIZED, AND OVARIECTOMIZED-ADRENALECTOMIZED FEMALE MONKEYS

(Urinary Extract Derived from 20 Hours of Urine Administered to Each Adrenalectomized Rat. Each Group of Rats Consisted of 9 to 11 Animals)

Monkey			Control rats	Urinary extract treated rats	Increase in mean survival %	P
Number	Weight in kilos	Status of animal	Mean survival hours	Mean survival hours		
2	5.2	intact	7.4 ± 0.28*	10.2 ± 1.16	38	0.05
			4.8 ± 0.58	10.8 ± 0.67	83	0.01
			4.6 ± 0.45	6.8 ± 0.87	48	0.05
	5.4	ovariectomized	5.9 ± 0.37	7.0 ± 0.61	19	0.05
			4.9 ± 0.40	6.7 ± 0.59	37	0.02
			4.9 ± 0.40	7.6 ± 0.66	57	0.01
	4.9	ovariectomized-adrenalectomized	6.8 ± 0.38	5.5 ± 0.36	−19	—
			5.3 ± 0.39	5.0 ± 0.36	− 6	—
6	4.5	intact	5.7 ± 0.44	6.2 ± 0.55	9	0.05
			4.8 ± 0.40	6.8 ± 0.82	42	0.05
			5.6 ± 0.27	8.8 ± 0.49	57	0.01
		ovariectomized	7.7 ± 0.29	8.1 ± 0.54	5	0.05
		ovariectomized-adrenalectomized	3.1 ± 0.26	3.1 ± 0.08	0	—
9	4.2	intact	3.1 ± 0.26	3.6 ± 0.23	16	0.03
		adrenalectomized	5.7 ± 0.44	3.9 ± 0.35	−32	—

* Standard Error.

and adrenalectomized female monkeys. Monkey #2 was studied in some detail. Cortin-like material was demonstrable when the animal was intact. Ovariectomy made little difference since two of the three urine samples collected after removal of the ovaries showed the presence of cold-protecting material. After adrenalectomy, no cortin-like material could be demonstrated in two different urine specimens.

TABLE 4. THE URINARY EXCRETION OF COLD-PROTECTING MATERIAL BY INTACT, CASTRATED, ADRENALECTOMIZED, AND CASTRATED-ADRENALECTOMIZED MALE MONKEYS
(Urinary Extract Derived from 20 Hours of Urine Administered to Each Adrenalectomized Rat. Each Group of Rats Consisted of 9 to 11 Animals)

Monkey			Control rats	Urinary extract treated rats	Increase in mean survival %	P
Number	Weight in kilos	Status of animal	Mean survival hours	Mean survival hours		
3	7.6	intact	4.9±0.40*	6.1±0.52	25	0.05
			5.7±0.44	6.0±0.37	5	>0.05
		castrated	7.7±0.29	7.1±0.61	− 8	—
			7.7±0.29	6.9±0.23	−10	—
4	6.9	intact	4.9±0.40	6.5±1.02	33	0.05
			5.7±0.44	6.1±0.58	7	>0.05
		castrated	5.7±0.49	7.4±0.53	30	0.05
			5.7±0.49	7.4±0.46	30	0.02
5	11.0	intact	4.9±0.40	6.8±0.66	39	0.05
			5.7±0.44	5.5±0.36	4	>0.05
			5.7±0.44	7.0±0.81	23	>0.05
		adrenalectomized	6.6±0.35	6.7±0.36	2	>0.05
8	6.0	intact	7.7±0.29	8.7±0.64	13	>0.05
		castrated	3.1±0.26	3.2±0.27	3	>0.05
		castrated-adrenalectomized	5.7±0.44	5.2±0.37	− 9	—

* Standard Error.

The results on Monkey #6 were not as consistent as those found for Monkey #2, since no cold protecting material could be demonstrated after ovariectomy. Only one assay was performed. Monkey #9 showed the presence of this material before adrenalectomy, but none after the operation.

As indicated in Table 4, urine samples from male Monkeys #3, #4, and #8 were assayed for cortin-like material before and after castration. Four samples were collected on Monkey #3, two before castration and two after the operation. Only one significant response was observed, that is, a 25 per cent increase before operation. Three positive responses were observed in the case of Monkey #4. Here, three of the four determinations showed significant increases. The smallest in-

crease (not significant) was found in one of the pre-operative samples. The urine of Monkey #8 showed no significant response either before or after castration.

Monkey #5 was adrenalectomized and Monkey #8 was adrenalectomized and castrated. Cortin-like material was demonstrated in the urine of Monkey #5 only once out of three trials before adrenalectomy. None was found in the one sample after adrenalectomy. The urine of Monkey #8 was negative before operation, after castration, and after adrenalectomy and castration.

Table 5 is a summary of the significance of responses obtained on intact, gonadectomized, gonadectomized-adrenalectomized, and adrenalectomized male and female monkeys. Of the twenty-one assays on the urine from intact animals (males and females), eleven showed significant positive responses. Of the nine urinary assays after gonadectomy, four were positive. However, after gonadectomy and adrenalectomy, or adrenalectomy alone, all six determinations on the urines from five animals were negative.

TABLE 5. SUMMARY OF SIGNIFICANT COLD-PROTECTING RESPONSES

Status of Monkey	Male monkeys		Female monkeys	
	Significant responses $P<0.05$	No significant responses $P>0.05$	Significant responses	No significant responses
intact	5	6	6	4
gonadectomized	2	3	2	2
gonadectomized-adrenalectomized	0	1	0	3
adrenalectomized	0	1	0	1

Since no cortin-like material could be demonstrated in the urine of adrenalectomized animals, it was evident that these animals could serve for the study of urinary recoveries after the administration of adrenal cortical extracts and desoxycorticosterone acetate.

These experiments are summarized in Table 6. In only one instance was urinary cortin-like material detected after the administration of desoxycorticosterone acetate. When Monkey #5 received a total of 22.5 mg. of desoxycorticosterone acetate,[2] the equivalent of as little as 5.4 hours of urine per assay animal showed a significant increase in mean survival of 34 per cent. Urine collected prior to treatment and administered in quantities equivalent to twenty hours of urine gave a negative response. Administration of 54 and 90 mg. of desoxycorticosterone acetate, respectively, to female monkeys #2 and #6 gave negative responses.

In six experiments, adrenal cortical extract[3] was administered and

[2] We wish to thank Ciba Pharmaceutical Products, Inc. for the generous supply of desoxycorticosterone acetate.

[3] Commercial Wilson Adrenal Cortical extract, 1 cc. = 75. gr. of adrenal tissue.

the urine tested for the presence of adrenal cortin-like material. Monkey #2 received adrenal cortical extract on two occasions. In the first experiment, 37 cc. of adrenal cortical extract was injected intravenously and an additional 4.5 cc. of extract was injected subcutaneously over a 48 hour period. The presence of cold-protecting material in the urine collected during this period was definite. The equivalent of 3.6 hours of urine gave an increase of 58 per cent in mean survival.

TABLE 6. THE URINARY EXCRETION OF COLD-PROTECTING MATERIAL AFTER THE ADMINISTRATION OF ADRENAL CORTICAL EXTRACT AND DESOXYCORTICOSTERONE ACETATE TO ADRENALECTOMIZED MONKEYS
(Each Group of Rats Consisted of 10 Animals)

Monkey		Treatment		Control Rats	Urinary extract equivalent administered per rat hours	Urinary extract treated rats mean survival hours	Increase in mean survival %	P
Number and sex	Status of animal	DOCA IM mg.	Adrenal cortical extract IV cc.	Mean survival hours				
2 ♀	Ovariectomized Adrenalectomized	0	0	6.8 ± 0.38*	20	5.5 ± 0.36	−19	—
		0	0	5.3 ± 0.39	20	5.0 ± 0.36	− 6	—
		0	37**	5.3 ± 0.39	3.6	8.4 ± 0.52	58	0.01
		0	45	5.3 ± 0.39	3.6	7.1 ± 0.75	34	0.05
		54	0	6.7 ± 0.55	7.2	7.1 ± 0.55	6	>0.05
5 ♂	Adrenalectomized	0	0	6.6 ± 0.38	20	6.7 ± 0.37	2	>0.05
		4	35	6.6 ± 0.38	8	8.5 ± 0.31	29	0.01
		0	45	6.1 ± 0.45	3.6	8.6 ± 0.57	41	0.01
		22.5	0	6.1 ± 0.45	5.4	8.2 ± 0.37	34	0.01
6 ♀	Ovariectomized Adrenalectomized	0	0	3.1 ± 0.26	20	3.1 ± 0.08	0	—
		0	47.5	6.1 ± 0.18	3.6	7.4 ± 0.33	21	0.01
		0	75	6.1 ± 0.18	3.0	7.5 ± 0.43	23	0.01
		90	0	5.7 ± 0.13	5.4	6.2 ± 0.27	9	>0.05

* Standard Error.
** 4.5 cc. of adrenal cortical extract was administered subcutaneously in addition to 37 cc. intravenously.

In a second experiment, Monkey #2 received 45 cc. of adrenal cortical extract during a two-day period and the urine collected during this period again contained cortin-like material. Thus the urinary extract equivalent to 3.6 hours produced an increase in mean survival of 34 per cent.

An adrenalectomized monkey, #5, received 35 cc. of adrenal cortical extract intravenously and 4 mg. of desoxycorticosterone acetate intramuscularly. Here again a positive response for adrenal cortical-like material was observed, a mean survival of 29 per cent above that

found for the control group. When 45 cc. of adrenal cortical extract was administered intravenously to the same animal, a response of 41 per cent was noted.

Two additional experiments on a second ovariectomized-adrenalectomized monkey, #6, showed similar excretions of active material after intravenous administration of 47.5 cc. and 75 cc. of adrenal cortical extract. Responses of 21 and 23 per cent, respectively, both significant increases were found.

DISCUSSION

It is clear from these experiments that normal adult male and female monkeys excrete material which protects adrenalectomized rats from low environmental temperatures. That this urinary cortin-like material is derived from the adrenal gland is strongly suggested since no active material could be demonstrated after bilateral adrenalectomy. Cortin-like material was demonstrable after gonadectomy.

The concentration of cortin-like material in the urine may serve as a specific index of adrenal cortical function. The determination of ketosteroids which has been used for this purpose is less specific since the gonads also may contribute to the total ketosteroid values.

SUMMARY

Urinary cortin-like material has been studied in intact, gonadectomized, adrenalectomized, and gonadectomized-adrenalectomized monkeys. Nine monkeys were studied. This biologically active material was detected by means of its ability to protect the adrenalectomized rat against low environmental temperatures. The presence of urinary cortin-like material was demonstrated in about half of the assays attempted for intact or gonadectomized male or female monkeys. After adrenalectomy, no positive assays were obtained in any instance either in males or females.

The administration of adrenal cortical extracts to adrenalectomized monkeys invariably led to the excretion of significant amounts of cortin-like material. In only one instance was urinary cortin-like material demonstrable after the administration of desoxycorticosterone acetate to adrenalectomized monkeys.

These results strongly suggest that the urinary cortin-like material has its origin in the adrenal gland.

REFERENCES

DORFMAN, R. I., AND B. N. HORWITT: *Federation Proc.* 2: 60. 1943.
DORFMAN, R. I., B. N. HORWITT AND W. R. FISH: *Science* 96: 496. 1942.
HORWITT, B. N., AND R. I. DORFMAN: *Science* 97: 337. 1943.
SCHILLER, S., AND R. I. DORFMAN: *Endocrinology* 33: 402. 1943.
SHIPLEY, R. A., R. I. DORFMAN AND B. N. HORWITT: *Am. J. Physiol.* 139: 742. 1943.
VENNING, E. H., M. M. HOFFMAN AND J. S. L. BROWNE: *J. Biol. Chem.* 148: 455. 1943.
WEIL, P., AND J. S. L. BROWNE: *Science* 90: 445. 1939.

METABOLISM OF THE STEROID HORMONES: THE METABOLISM OF TESTOSTERONE IN THE MALE CHIMPANZEE[1]

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IN A PREVIOUS INVESTIGATION (Fish, Young and Dorfman, 1941), it was reported that the urinary concentrations of androgenic substances excreted by male chimpanzees were only about 10 per cent as high as that reported for men (Gallagher, Peterson, Dorfman, Kenyon and Koch, 1937). This difference is of interest, especially in view of the close phylogenetic relation of the chimpanzee to man. This study is concerned with the metabolism of testosterone in male chimpanzees compared to that already described for men. Further, if the metabolism of testosterone in the chimpanzee could be shown to be qualitatively similar to that in man a valuable test animal could be made available for further metabolism studies.

Callow (1939) and Dorfman, Cook and Hamilton (1939) have shown that the principal urinary metabolites of testosterone in men are androsterone and etiocholanol-3 (α)-one-17. The conversion of testosterone to androsterone has also been demonstrated in the pregnant monkey (Horwitt, Dorfman and van Wagenen (1944). Isoandrosterone has been shown to be a metabolite of testosterone in men (Dorfman, 1941) and in male guinea pigs (Dorfman and Fish, 1940).

EXPERIMENTAL

Three hundred mg. of testosterone propionate[2] (equivalent to 250 mg. of testosterone) were administered orally each day for five days

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[2] Testosterone propionate was kindly supplied by the Ciba Pharmaceutical Products, Inc. under the trade name Perandren.

to each of two adult male chimpanzees. During the period of administration and for the following four days, the urine was quantitatively collected under toluene. All the specimens were pooled and amounted to 21 liters. To each 100 cc. of urine was added 10 cc. of concentrated hydrochloric acid at the time of shipment from Orange Park, Florida to New Haven, Connecticut, where chemical examination of the urine was begun. Hydrolysis and extraction with benzene were carried out simultaneously by a modification of the method of Dingemanse, Borchardt and Laqueur (1937), described in Paper II of this series (Fish and Dorfman, 1942). The compounds soluble in benzene were partitioned between ether and 10 per cent aqueous sodium hydroxide. The alkaline solution was separated and extracted once with ether, which was added to the alkali-insoluble (neutral) fraction. The ether containing compounds were separated into ketonic and non-ketonic fractions by means of the Girard-Sandulesco reagent, trimethylacethydrazide ammonium chloride, according to the procedure previously described (Dorfman, Cook and Hamilton, 1939). The neutral ketones were dissolved in 20 cc. of 90 per cent ethanol. To this solution was added 20 cc. of a 2 per cent solution of digitonin in 90 per cent ethanol. After standing over-night, a small amount of precipitate separated and was removed by centrifugation. The precipitate was washed with 90 per cent ethanol and ether, the washings being added to the supernatant solution containing the digitonin-non-precipitable compounds. The latter solution was evaporated to dryness. The dried residue was dissolved in 5 cc. of pyridine and heated on the steam bath for 60 minutes. The pyridine was then taken up in eight times its volume of ether and the precipitated digitonin separated by centrifugation. The digitonin was washed with ether, which was added to the supernantant fluid. The combined ether solutions were washed successively with 10 per cent hydrochloric acid, 10 per cent aqueous sodium hydroxide and water. The ether was evaporated and the residue dried by distilling absolute ethanol from it under reduced pressure.

The ketonic digitonin non-precipitable fraction was dissolved in 30 cc. of carbon tetrachloride and absorbed on a column of Brockmann's aluminum oxide. Elutions were carried out as indicated in table 1.

Isolation of Androstenone-17

The viscous yellow oil derived from fractions 1 and 2 (table 1) were combined and sublimed in high vacuum up to 106° C. The first sublimate was recrystallized three times from absolute methanol to constant melting point, 100–102° C.[3] The weight of this material was 95 mg. Further sublimation yielded another 23 mg. of a compound

[3] Melting points were determined by means of the Fischer-Johns melting point apparatus.

which melted at 99–101° C. A melting point of a mixture of these two compounds melted at 100–102° C. Thus it appeared that the two compounds were identical and the total yield was 118 mg.

An attempt to form an acetate yielded a substance, melting point

TABLE 1. ADSORPTION AND ELUTION OF NEUTRAL KETONIC DIGITONIN NON-PRECIPITABLE FRACTION

Fraction number	Solvent	Volume cc.	Remarks
1	CCl_4	30	Viscous yellow oil
2	CCl_4	50	Viscous yellow oil
3– 9	CCl_4	350	Crystalline material
10	0.1% Ethanol in CCl_4	50	Trace of colorless oil
11	0.2% Ethanol in CCl_4	50	Trace of colorless oil
12	0.3% Ethanol in CCl_4	50	Trace of colorless oil
13–14	3.0% Ethanol in CCl_4	100	Red-orange oil
15–16	5.0% Ethanol in CCl_4	150	Red-orange oil

103–105° C. and when mixed with the original material (m.p. 100–102° C.) melted at 100–103° C.

Analysis: Calculated for androstenone-17

	C	H
Theoretical ($C_{19}H_{28}O$)	83.76	10.37
Found:	83.29	10.37
	83.12	10.32

A sample of androstenone-17,[4] melting point 95–98° C., was mixed with a sample of our compound, the mixture melted at 95–98° C.

The compound was found to possess androgenic activity equivalent to one-eleventh of that found for androsterone. Similar androgenic activity was found for an authentic sample of androstenone-17.

Isolation of Androsterone

Fractions 3 to 9 yielded on recrystallization successively from methanol, ethanol, and acetone yielded 54 mg. of androsterone. Three crops of androsterone crystals were isolated; namely, 6 mg. (melting point 180–182° C.), 15 mg. (melting point 180–182°C.), and 33 mg. (melting point 178–179° C.). A mixture of an authentic sample of, androsterone, melting point 178–179° C., with an isolated sample, melting point 180–182° C., melted at 178–179° C. An acetate melted at 159–161° C. and when mixed with an authentic sample of androsterone acetate, melting point 159–160° C., melted at 159–161° C.

Isolation of Etiocholanol-3 (α)-17-one

Fractions 13–16 were combined and sublimed at 110° C. The sublimate was recrystallized repeatedly from absolute methanol until

[4] Kindly supplied by Dr. Pearlman of Clark University.

free from color. The material melted at 118–125° C. and after four recrystallizations from aqueous methanol melted at 123–128° C. This was rechromatographed as represented in table 2.

The residues of fractions F, G, H (table 2) were combined and rechromatographed. Sub-fraction from 0.3% ethanol in carbon tetrachloride yielded after three recrystallizations 20.5 mg. of material m.p. 142–144° C. When mixed with an authentic sample of etiocholanol-3 (α)-17-one (m.p. 136–137° C.), the mixture melted at 142–

TABLE 2. ADSORPTION AND ELUTIONS OF FRACTIONS 13–16

Fraction	Solvent	Volume cc.	Remarks
A, B, C	CCl_4	150	Yellow oil
D	0.1% Ethanol in CCl_4	50	Yellow oil
E	0.2% Ethanol in CCl_4	50	Nothing
F	0.3% Ethanol in CCl_4	50	Semi-crystalline material and red pigment
G	0.3% Ethanol in CCl_4	50	Semi-crystalline material and red pigment
H	0.3% Ethanol in CCl_4	50	Semi-crystalline material and red pigment
I	0.3% Ethanol in CCl_4	50	Clear oil
G	0.4% Ethanol in CCl_4	100	Nothing
H	1.0% Ethanol in CCl_4	50	Nothing

TABLE 3. METABOLITES OF TESTOSTERONE IN THE MALE CHIMPANZEE
(Three Grams of Testosterone Propionate Administered Orally)

Substance or material	Amount	% of Ketosteroids	% of Androgens
Ketosteroids (ketonic fraction)	1050 mg.	—	—
Androgens (ketonic fraction)	1730 I.U.	16.5 (calculated as androsterone)	—
Androstenone	54 mg.	5.1	31.3
Androstenone-17	118 mg.	11.2	6.2
Etiocholanol-3 (α)-17-one	20.5 mg.	2.0	—

144° C. The benzoate melted at 155–157° C. and when mixed with an authentic sample of etiocholanol-3 (α)-17-one benzoate (m.p. 159–160° C.), the melting point was 157–159° C.

DISCUSSION AND CONCLUSIONS

After the administration of testosterone propionate to male chimpanzees, androstenone-17, androsterone and etiocholanol-3 (α)-17-one were isolated from the urine. The latter two 17-ketosteroids have been recognized previously as urinary metabolites of testosterone in men. Androstenone-17 probably represents a dehydration product of androsterone formed during the hydrolysis and extraction of the urines.

During pre-treatment periods of urine collections, Pan excreted an average of 5.8 I.U. per day and Bokar excreted an average of 8.2 I.U. per day. If we assume that the administration of testosterone propionate had no effect on the endogenous production of androgens, the

total androgenic excretion due to endogenous hormones should be 126 I.U. After the administration of 1500 mg. of testosterone propionate to each of the two animals over a five day period with urine collections continuing for an additional four days, a total of 1730 I.U. of androgenic material was found. Thus the testosterone caused an increase of at least thirteen-fold.

Of the 1730 I.U. of androgenic material contained in the ketonic fraction, a total of 37.5% was accounted for by direct isolation. Fifty-four mg. of androsterone represented 31.3% of the biologically active material while androstenone-17 represented 6.2% of the androgens (table 3).

Of the 1050 mg. of 17-ketosteroids excreted, a total of 18.3% was isolated and identified. Androsterone represented 5.1% while androstenone-17 and etiocholanol-3 (α)-17-one accounted for 11.2% and 2.0% respectively, of the ketosteroids.

If we assume that the isolated androstenone-17 arose by the dehydration of androsterone then the actual amount of androsterone is considerably increased.

SUMMARY

The oral administration of 1.5 gms. of testosterone propionate to each of two adult male chimpanzees produced a thirteen-fold increase in urinary androgenic substances.

Androsterone and etiocholanol-3 (α)-17-one have been identified as urinary metabolites of testosterone in the male chimpanzee. This finding is in accord with previous studies on men. Androstenone-17 was also isolated from the urinary extracts but this substance is considered to be formed by dehydration of androsterone during the hydrolysis and extraction of the urine.

REFERENCES

CALLOW, N. H.: *Biochem. J.* 33: 559. 1939.
DINGEMANSE, E., H. BORCHARDT AND E. LAQUEUR: *Biochem J.* 31: 500. 1937.
DORFMAN, R. I.: *Proc. Soc. Exper. Biol. & Med.* 46: 351. 1941.
DORFMAN, R. I., J. W. COOK AND J. B. HAMILTON: *J. Biol. Chem.* 130: 285. 1939.
DORFMAN, R. I., AND W. R. FISH: *J. Biol. Chem.* 135: 349. 1940.
FISH, W. R., AND R. I. DORFMAN: *J. Biol. Chem.* 143: 15. 1942.
FISH, W. R., W. C. YOUNG AND R. I. DORFMAN: *Endocrinology* 28: 585. 1941.
GALLAGHER, T. F., D. H. PETERSON, R. I. DORFMAN, A. T. KENYON AND F. C. KOCH: *J. Clin. Investigation* 16: 695. 1937.
HORWITT, B. N., R. I. DORFMAN AND G. VAN WAGENEN: *Endocrinology.* (In press.)

EXPERIMENTAL HYPOTHYROIDISM IN THE MONKEY[1]

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THE EFFECTS of thyroid removal in animals are well known, and in general agree with the clinical state of hypothyroidism. It has been established that hypercholesterolemia accompanies hypothyroidism in man, and the measurement of the cholesterol concentration of the serum is used routinely as an aid in the diagnosis of this disease. Complete thyroidectomy, done for relief of intractable anginal pain and congestive heart failure (Gilligan, *et al.*, 1934; and Schnitker, *et al.*, 1936) causes a rise in serum cholesterol. Nothing is known concerning the mechanism of this process.

In adult animals, the relationship between hypothyroidism and serum cholesterol is not as close as it is in the clinical condition. In the dog, a definite rise follows thyroidectomy (Thompson and Long, 1941; Chaikoff, *et al.*, 1941; and Parhon and Dérévici, 1926). Fleischmann, Shumacker and Wilkins, 1940, and Rémond, *et al.*, 1924, found an increase in the rabbit. On the other hand, Turner, Present, and Bidwell, 1938, were unable to demonstrate hypercholesterolemia in thyroidectomized rabbits unless the animals were on a high cholesterol diet.

Schmidt and Hughes, 1938, have shown that in the dog thyroxine ingestion does not cause a decrease in serum cholesterol unless the animal has been thyroidectomized.

The clinical picture of adult myxedema has not been duplicated in animals (Vincent, 1925), although cretinism in the immature animal has been demonstrated in many species. The effect of thyroidectomy on weight changes in adult animals has not been thoroughly investigated.

There is more unanimity of opinion regarding creatine retention during hypothyroidism. Thorn, 1936, found an increase in creatine tolerance during clinical hypothyroidism, but in the young child the correlation is not as close (Wilkins and Fleischmann, 1941).

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Fleischmann, *et al.*, 1943, reported that no significant change occurred in the serum cholesterol concentration of adult female thyroidectomized monkeys, although young animals did show an increase. The present experiments were designed to re-examine this condition in adult monkeys and to explore the usefulness of other tests as indicators of hypothyroidism in this species.

METHODS AND MATERIALS

Ten adult female monkeys (*Macaca mulatta*) weighing 4650–5600 gms., consisting of 3 normals and 7 spayed, were used in this study, each operated animal also serving as its own preoperative control. Seven were thyroidectomized and 3 remained as unoperated controls. Thyroidectomies were done under nembutal and ether anaesthesia using aseptic technique. The thyroid gland was exposed, the isthmus divided, and each lobe was removed separately by blunt dissection. Some hemorrhage may occur from the superior thyroid artery which enters the thyroid gland very deeply but the inferior thyroid artery is easily exposed. These were ligated with silk sutures. The two external parathyroid glands were carefully dissected free from thyroid tissue and reimplanted into the sternohyoid muscle. Four to five days after thyroidectomy, the animals exhibited hyperirritability, tetanic convulsions and even collapse. They were given parathyroid hormone subcutaneously and calcium gluconate intravenously. This was necessary for only a few days, after which these manifestations were no longer noted. One animal died in convulsions 3 months after thyroidectomy.

Cholesterol determinations were made according to the method of Schoenheimer and Sperry (1934) as modified by Sperry (1938). Creatinine and creatine were determined in duplicate by the Folin-Wu method, and creatine tolerance tests were carried out according to a method previously described (Jailer, 1940). A total of 748 cholesterol and 433 creatine determinations were done.

Circulation times were obtained by the NaCN method of Lowenhart, *et al.* (1922), and of Robb and Weiss (1933). An injection of 0.07 cc. of a 1% NaCN solution in saline was made into the posterior popliteal vein. This was invariably followed by a sudden gasping of breath. The interval between injection and response was timed by a stop watch calibrated in 0.1 seconds.

The animals were fed their normal laboratory diet, which is essentially creatine-free, consisting of vegetables, bread, grain, and occasionally eggs. They were weighed at various intervals.

At necropsy, serial sections of the thyroid bed and adjacent structures were made and examined microscopically. Small areas of tissue which might prove to be thyroid were sectioned serially. Other large blocks, at the site of operation, including the laryngeal cartilages and neighboring soft parts were serially sectioned at 10μ. Every fifteenth section was mounted and stained. Thus both sides of the entire larynx and trachea at the operative site were sectioned and one section in every 150μ was examined microscopically for remnants. In a search for aberrant thyroid tissue, the area of the foramen caecum of the tongue, and contents of the superior mediastinum and the thymus were sectioned. In each instance the muscular site of implantation of the parathyroids was sectioned serially.

Pertinent data regarding survival and autopsy findings are presented in table 1. In 3 of 7 animals no thyroid tissue could be found. In three of the remaining 4, thyroid tissue was found associated with the parathyroid gland in the muscle at the site of the translocated gland. Although the usual care had been taken to free the delicate capsule of the parathyroids of any thyroid tissue, these fragments were carried with the transplant. They were minute, being at most 1 mm. in maximal diameter. One monkey had a small fragment, about 1 mm. in diameter in the operative bed on the larynx. This may

TABLE 1. THYROID EXCISION IN MONKEYS

Monkey	Accession date wt. gms.	Thyroidectomy date wt. gms.	Autopsy date wt. gms.	Postoperative survival months	Thyroid status
627 ♀ *	10-26-39 5000	6-30-41 4750	4-10-42 4900	10	No thyroid
595 ♀ Ovariectomized	9-18-39 4000	11- 6-41 4300	5- 6-43 3940	18	No thyroid
614 ♀ ** Ovariectomized	10-26-39 5150	11-27-41 4900	2- 6-42 4900	3	No thyroid
613 ♀ Ovariectomized	10-26-39 4570	7- 1-41 4790	4-29-43 6000	22	Fragment with parathyroid Hyperplastic epithelium
601 ♀ Ovariectomized	10-18-39 4600	3- 3-42 5050	3-29-43 5000	13	Fragment with parathyroid No hyperplasia
594 ♀ Ovariectomized	10-10-39 4140	10-30-41 5600	5-11-43 5930	19	Fragment with parathyroid Hyperplastic epithelium
653 ♀ Ovariectomized Postoperative	12- 8-39 4000	10-30-41 4850	3-30-43 5000	17	Fragment thyroid bed Slight hyperplasia: not uniform

* Developed tuberculosis and was killed.
** Died in tetanic convulsions.

have been a fragmented piece which was torn off and accidentally reimplanted at the operation. It was hardly large enough to be a remnant left in situ at operation. The epithelium of the thyroid in three of the fragments was hyperplastic. No true accessory thyroid tissue was found.

In one animal (614) the transplanted parathyroid gland was functional for a time, but convulsions occurred three months postoperative and restorative therapy did not prevent the death of the animal. Serum calcium determinations were not made.

RESULTS

Weight Changes

Six of the thyroidectomized animals were weighed at irregular intervals during a period of some months before and during the entire period after thyroidectomy. The weights of other monkeys in the colony are also presented for comparison. Except in one instance, the weight changes of the hypothyroid monkeys are not highly significant

(table 1). The only spectacular gain in weight occurred in monkey 613. When received in the laboratory in October, 1939, she weighed 4.5 kg. During the 20 months preceding thyroid operation she had gained at most 400 gms. Within 60 days after thyroidectomy the weight increased from 4790 gms. to 6330 gms., a gain of 1.5 kg. This weight was maintained with a monthly gain or loss of less than 300 gms. for the following 22 months.

Weight records of other unoperated monkeys kept under the same conditions show some animals maintaining the same weight over several years. A normal monkey, No. 605 ♀, has not varied from 7.0 kg. by more than 300 gms. for 4½ years. Another colony monkey, 617 ♀, gained 0.7 kg. in one month and 2.3 kg. in one year to 8.7 kg. No constant weight change can be ascribed to the hypothyroid state of the monkeys reported here, with the possible exception of monkey 613.

Two animals with thyroid remnants, 594 and 613, were the only ones which showed clinical signs of myxedema. There was an increase in weight; they had coarse, dry scaly skin, loss of hair (this was especially true of 594), protuberant abdomen, and heavy, oedematous lower eyelids. Although their skin was especially thick, there was no pitting edema. The clinical signs of myxedema could not be demonstrated by microscopic examination of the dermis and they did not become more sluggish in behavior. The remaining monkeys of this series could not be distinguished from the normal monkeys in the colony.

Circulation Time

The average for 14 leg-to-carotid sinus circulation times in 7 different normal animals was 6.0 seconds (with a range of 4.5–6.8 seconds). After thyroidectomy, the average time was 7.8 for 20 tests with 6 different monkeys (a range of 6.2–10.5 seconds). In 3 of the animals, circulation times were determined both preoperatively and at various times postoperatively, with an average difference of 2.7 seconds. Except for one record on monkey 613, which was 6.2 seconds postoperatively, all the results show a definite slowing of the circulation after thyroidectomy. When this method was checked on the same day by the same persons, using first one leg and then the other, duplicates within 0.2 seconds have been obtained.

Creatinuria

The animals were kept on a creatine free diet and the average amount of urinary creatine excreted during the control period was ascertained. One month after thyroidectomy, a similar procedure was followed. The difference in excretion was not marked, the monkeys showing a slight decrease in creatinuria. However, thyroidectomy increased the capacity to retain exogenous creatine as shown by creatine-retention tests, table 2.

TABLE 2. CREATINE RETENTION TESTS IN HYPOTHYROID MONKEYS
Results for two tests are given with 1) 200 mgs., and 2) 400 mgs. exogenous creatine administered.

Monkey	Preoperative % creatine excreted	1–2 Months postoperative % creatine excreted
613	1) 49 2) 89	43 60
614	1) 41 2) 49	8 14
627	1) 32 2) 86	0 0
653	1) 80 2) 81	22 51

Creatine excretion determinations made 5 to 10 months postoperatively showed that the levels had returned to the preoperative base line. No creatine-retention tests were done at this time.

When the over-all average creatinine excretion levels were examined it was evident that creatine excretion did not change constantly or significantly throughout the entire course of the experiment.

Cholesterol

A series of preoperative serum cholesterol determinations were performed on all the animals over periods of time ranging from 1 week to 4 months. The animals were then thyroidectomized and examined again at intervals during a period of 10 to 22 months.

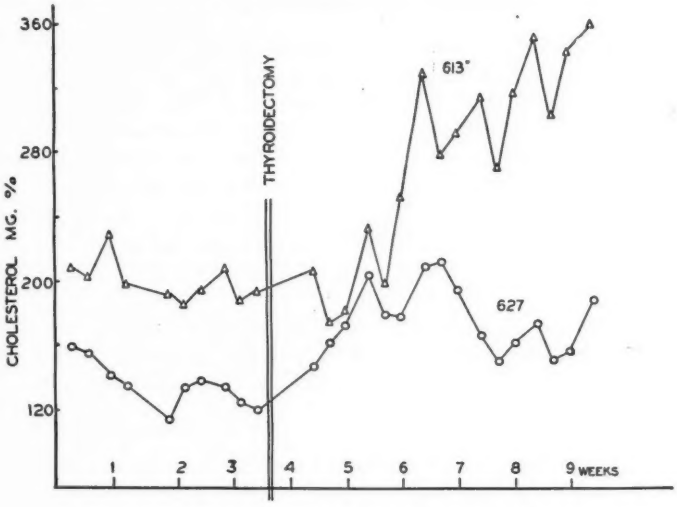

FIG. 1. The effect of thyroidectomy on serum cholesterol.

The results after thyroidectomy were variable. In no animal did the total cholesterol concentration rise to the extent reported in the dog, rabbit, or the human. Monkey 613, which had a thyroid fragment, showed the greatest rise: from about 200 mg. per 100 cc. to more than 350 mg. per 100 cc. in a period of a month (fig. 1). This was during the time of rapid increase in body weight. Nine months

TABLE 3. CHOLESTEROL CONCENTRATION IN BLOOD SERUM OF HYPOTHYROID MONKEYS

All of the data represent total cholesterol concentration in mg. per 100 cc. They were selected from the continuous record to show the values at the time proximal to the stated interval.

	613 ♀	627 ♀	653 ♀	594 ♀	595 ♀	601 ♀
preop.						
2 wks.	195	142	140	190	154	162
1 wk.	194	139		180	138	158
postop.						
1 wk.	207	148	136	166	126	161
2 wks.	234	180	145	200	135	194
3 wks.	330	210	171	249	144	211
1 mo.	318	162	154	196	151	184
2 mos.	307	195	177	196	134	160
3 mos	307	178	191	175	122	160
4 mos.	242	150	177	158	136	175
5 mos.	259	217	156	220	153	
6 mos.	234	218	152	178	141	
7 mos.	206	170	168	189	120	
8 mos.	258	179	158	200	176	
9 mos.	281		139	175		
10 mos.	230					184
11 mos.	281					162
12 mos.	300					
14 mos.			202	218	194	
15 mos.			213	181	174	

postoperatively, the serum cholesterol level had declined toward the preoperative value and varied around 240 mg. per 100 cc.

Monkeys 627 and 614, which were completely thyroidectomized, and 594 and 601, which had thyroid tissue, all showed a slight but definite rise in cholesterol during one month, but there was a slow return to the normal during two additional months. Monkey 595, which was completely thyroidectomized exhibited no change in cholesterol level. Monkey 653, which had a thyroid remnant, showed an increase to 200 mg. per 100 cc. only after 14 months of postoperative life.

Cholesterol determinations made at various intervals for as long as $1\frac{1}{2}$ years postoperatively showed that the values remained within the range found three months after thyroidectomy, table 3.

Effect of Thyroxine

In this experiment, 2 thyroidectomized and 2 normal animals were each injected with 0.25 mg. thyroxine daily for 10 days. Each responded with a creatinuria which increased to 100 mg. per day, a

result similar to that obtained previously (Jailer, 1941). All 4 animals responded with a fall in serum cholesterol to values as low as 80 mg. per 100 cc. (Fig. 2). There was, however, no difference between the normal and thyroidectomized animals in the magnitude of the response.

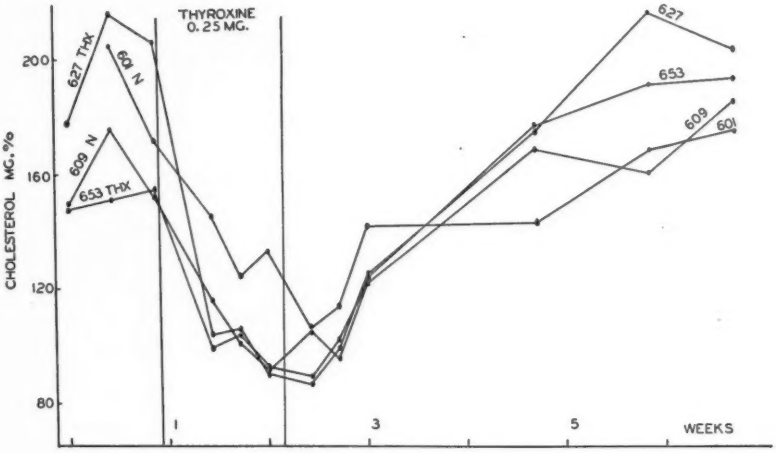

FIG. 2. The effect of 0.25 mg. thyroxine on serum cholesterol.
THX = thyroidectomized
N = normal

Effect of Thyrotropic Hormone

The injection of thyrotropic hormone was used as a check for completeness of thyroidectomy. Thyrotropic hormone stimulates the thyroid. If no response is obtained, it may be assumed that little or no thyroid tissue is present.

Two normal monkeys (numbers 593 and 605), which were injected daily for 7 days with 50 mg. of an extract of beef anterior pituitary gland containing the thyrotropic activity responded with an increased creatinuria and a decrease in serum cholesterol.

Five operated animals were then treated with the same daily dose of the same extract, but for 10 days, and the urinary creatine and the serum cholesterol values were determined. Monkeys 613 and 653 responded with a decrease in cholesterol and a decided increase in creatinuria, indicating the presence of some thyroid tissue which was stimulated (Fig. 3). This was confirmed at autopsy. In 613, a fragment of thyroid was found in the sternothyroid muscle with the transplanted parathyroid. Monkey 653 had a small 1 mm. fragment at the operative site on the left of the trachea. Monkeys 594 and 595 showed neither a rise in creatinuria nor a decrease in serum cholesterol, al-

though 594 had a nest of thyroid follicles attached to the transplanted parathyroid at autopsy. Monkey 601 responded by exhibiting no creatinuria but a fall in serum cholesterol from 160 mg. to 100 mg. per 100 cc. This is difficult to interpret since creatine response is usually much more sensitive to thyroid administration than changes in cholesterol. Creatinuria will increase on small doses of thyroxine (0.1 mg., 0.25mg., and 0.5 mg.) without affecting serum cholesterol levels.

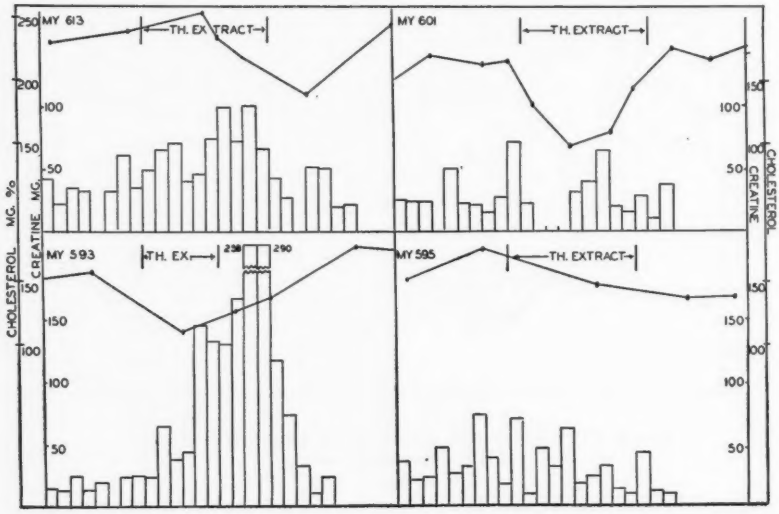

FIG. 3. The effect of thyrotropic hormone on serum cholesterol and urinary creatine. Line graph = cholesterol values. Bar graph = urinary creatine. Each bar represents a 24 hour specimen.

MY. 593 — normal control
TH. EX. = thyrotropic hormone

A small 1.0 mm. non-hyperplastic fragment of thyroid was associated with the parathyroid in this animal.

An interesting phenomenon occurred in the 2 normal monkeys treated with thyrotropic hormone. After cessation of the thyrotropic injections the cholesterol levels gradually returned to the base line and then proceeded to rise above the level of the preinjection period, not returning to that level until about 1 month after cessation of treatment. The phenomenon of overshooting the original base line after cessation of treatment did not occur in the animals when thyroxine was given instead of thyrotropic hormone.

DISCUSSION

Seven adult female monkeys were operated on and the thyroid and parathyroid glands were removed. One or two parathyroid glands were transplanted. in each animal, at the time of operation.

Each of the monkeys showed convulsive seizures within 4 to 6 days postoperative, but were maintained for a time with a parathyroid preparation and calcium gluconate. After autopsy, at least one parathyroid gland was found at the site of implantation in all but one of the monkeys, which died 3 months postoperatively.

No thyroid tissue was found in 3 animals after an extensive microscopic examination. In one, a fragment was found in the thyroid bed, and in 3 monkeys thyroid tissue was found in association with the parathyroid transplant. These experiments deal, therefore, with hypothyroid monkeys rather than thyroidectomized animals. However, the thyroid status of each animal is known.

In their report on thyroidectomy in rhesus monkeys, Fleischmann, Shumacker and Straus (1943) state that "no effort was made to preserve the parathyroids." As in our group, four of their animals developed convulsions which were relieved by parathyroid hormone and calcium gluconate. These investigators made microscopic examination of tissue suggestive of thyroid tissue, but in only one animal was a systematic examination of all possible thyroid-bearing tissue made. These authors make no suggestion as to the mechanism of calcium-phosphorus balance in these monkeys in the absence of the parathyroid glands.

These authors concluded that thyroidectomy has no effect on the serum cholesterol concentration in adult monkeys, though it does induce an hypercholesterolemia in young monkeys. This result is of considerable interest because of its contrast with the findings in other species, particularly man, in which hypothyroidism is associated as a general rule with an increased level of serum cholesterol. In the present investigation an unmistakable and sustained hypercholesterolemia followed thyroidectomy in one adult ovariectomized monkey (number 613), even though a fragment of thyroid tissue was found at autopsy, and in four other adult monkeys there was a temporary increase well above the base line in the cholesterol concentration after operation.

The method (Pijoan and Walter, 1937) which Fleischmann, *et al.*, used for cholesterol estimation is open to criticism. It does not include a saponification step which has been shown to be essential for the accurate determination of total cholesterol of the blood serum by the usual Liebermann-Burchard color reaction (Sperry & Brand, 1943) because the rates of color development are different for cholesterol combined in esters and for free cholesterol. This difference becomes greater, the lower the temperature, and at 0 to 2° Reinhold (1935) found that only the combined cholesterol gives color within 45 to 50 minutes. Since in the method used by Fleischmann, *et al.*, the color is developed in unsaponified extracts at -5 to $+5°$ for 45 minutes, it may be presumed that they measured only the combined cholesterol.

Throughout our study both free and total cholesterol were determined. The proportion between the fractions, though somewhat more variable (Sperry and Jailer, 1944) than it is in human beings (Sperry, 1936), showed no consistent change during the rises in total cholesterol after thyroidectomy. It follows that even though the values for total cholesterol reported by Fleischmann, *et al.*, actually represented combined cholesterol, they should have reflected a postoperative hypercholesterolemia had it occurred in their adult monkeys.

The more likely explanation of the difference between the present results and those of Fleischmann is that occasional adult, thyroidectomized monkeys develop hypercholesterolemia for unknown reasons. Fleischmann, *et al.*, chanced to encounter no responsive adult animals in their series. Our findings are in accord with theirs in showing that adult thyroidectomized monkeys are, in comparison with other species, remarkably unresponsive in regard to changes in serum cholesterol concentration.

Because of the lack of increase in cholesterol, it was postulated that perhaps cholesterol metabolism in monkeys is not labile. However, thyroxine and thyrotropic hormone administration caused a significant decrease in the serum cholesterol level and egg-feeding and the ingestion of cholesterol caused a marked increase in the serum levels in both normal and thyroidectomized animals (Sperry and Jailer, 1944), so that this explanation is untenable. The diet of our animals consisted of fruits and vegetables containing sterols which the animal body cannot absorb (Schönheimer, 1929). An egg thrice weekly furnished the only dietary source of cholesterol except during the periods described by Sperry and Jailer.

Fleischmann, Shumacker and Straus (1943) found no difference in creatine excretion between normal and thyroidectomized monkeys. Our results showed a slight decrease one month postoperatively, but creatine excretion in female monkeys is small at any time. When the animals were given exogenous creatine (creatine retention tests) the difference became more marked and a thyroidless animal was able to retain more creatine than during the control periods. It appears that the differences in creatine metabolism are greater when the animals are given an overload of creatine.

The results obtained with the NaCN method indicate that the circulation time is slowed by about 30%. This lowered rate is maintained.

Two of the monkeys, numbers 613 and 594, developed the clinical picture of myxedema, which was characterized by a gain in weight, development of coarse thick skin, pendulous abdomen and edematous lower eyelids. Both monkeys had been ovariectomized about one year before the start of these experiments and each had thyroid fragments. However, this condition was not duplicated in other animals treated the same way. It is interesting to note that the greatest incidence of

human myxedema occurs at the menopause and it has been reported as developing after ovariectomy in the human being.

The foregoing observations indicate that thyroidectomy or a hypothyroid state in monkeys does not induce the marked or constant variations from the normal so frequently seen in the human being.

SUMMARY

The thyroid glands were removed from seven adult female monkeys. The parathyroids were dissected free and reimplanted.

The effects of hypothyroidism in these monkeys were slight as recorded over a postoperative period of 10 to 22 months.

Circulation time was decreased by approximately 30%.

Serum cholesterol increased markedly in only one animal. Urinary creatine did not change significantly, but there was an increased retention of exogenous, administered creatine.

REFERENCES

CHAIKOFF, I. L., C. ENTENMAN, G. W. CHANGUS AND F. L. REICHERT: *Endocrinology* 28: 797. 1941.

FLEISCHMANN, W., H. B. SHUMACKER, JR. AND L. WILKINS: *Am. J. Physiol.* 131: 317. 1940.

FLEISCHMANN, W., H. B. SHUMACKER, JR., AND W. L. STRAUS, JR.: *Federation Proc.* 1: 24. 1942.

FLEISCHMANN, W., H. B. SHUMACKER, JR. AND W. L. STRAUS, JR.: *Endocrinology* 32: 238. 1943.

GILLIGAN, D. R., M. C. VOLK, D. DAVIS AND H. L. BLUMGART: *Arch. Int. Med.* 54: 746. 1934.

LOEVENHART, A. S., B. H. SCHLOMOVITZ AND E. G. SEYBOLD: *J. Pharmacol. & Exper. Therap.* 19: 221. 1922–23.

PARHON, C.-I., AND H. DÉRÉVICI: *Compt. rend. Soc. de biol.* 95: 787. 1926.

PIJOAN, M., AND C. W. WALTER: *J. Lab. & Clin. Med.* 22: 968. 1937.

REINHOLD, J. G.: *Proc. Soc. Exper. Biol. & Med.* 32: 614. 1934–35.

RÉMOND, A., H. COLOMBIÉS AND J. BERNARDBEIG: *Compt. rend. Soc. de biol.* 91: 445. 1924.

ROBB, G. P., AND S. WEISS: *Am. Heart J.* 8: 650. 1933.

SCHMIDT, L. H., AND H. B. HUGHES: *Endocrinology* 22: 474. 1938.

SCHNITKER, M. T., L. H. VAN RAALTE AND E. C. CUTLER: *Arch. Int. Med.* 57: 857. 1936.

SCHOENHEIMER, R., AND W. M. SPERRY: *J. Biol. Chem.* 106: 745. 1934.

SCHÖNHEIMER, R.: *Zeitschr. f. physiol. Chem.* 180: 1. 1929.

SPERRY, W. M.: *J. Biol. Chem.* 114: 125. 1936.

SPERRY, W. M.: *Am. J. Clin. Path.* 2: 91. 1938.

SPERRY, W. M., AND F. C. BRAND: *J. Biol. Chem.* 150: 315. 1943.

THOMPSON, K. W., AND C. N. H. LONG: *Endocrinology* 28: 715. 1941.

THORN, G. W.: *Endocrinology* 20: 628. 1936.

TURNER, K. B., C. H. PRESENT, AND E. H. BIDWELL: *J. Exper. Med.* 67: 111. 1938.

VINCENT, S., AND W. A. JOLLY: *J. Physiol.* 32: 65. 1904.

WILKINS, L., AND W. FLEISCHMANN: *J.A.M.A.* 116: 2459. 1941.

THE INFLUENCE OF DIET ON THE CHOLESTEROL CONCENTRATION OF THE BLOOD SERUM IN NORMAL, SPAYED, AND HYPOTHYROID MONKEYS[1]

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THE INFLUENCE of dietary cholesterol on the concentration of cholesterol in the blood has been the subject of much study both in man and in experimental animals. Most investigators have failed to find any consistent effect of a single cholesterol-rich meal. Thus, Sperry (1936) reported that in ten experiments the average change in serum cholesterol concentration was zero during the three to four hours following the ingestion by healthy human subjects of a breakfast of three eggs, six slices of bacon, two slices of toast heavily buttered, two cups of coffee with cream, and orange juice. Fröhling (1936) reported that only in one instance did the administration of as much as 15 gm. cholesterol in olive oil have any effect on serum cholesterol concentration. Even in rabbits, in which large accumulations of cholesterol result from continued feeding, a single dose of cholesterol has been reported not to affect the concentration in the blood (Ssokoloff and Mjassnikow, 1923; Iljinsky, 1926–27).

There is less information concerning the effect of continued cholesterol feeding at a high level in omnivorous animals. Mjassnikow (1926) found little or no change in the cholesterol level of the blood serum following the feeding of eight eggs per day for four or ten days to normal, nephrotic, and arteriosclerotic human subjects. Wilkins, Fleischmann, and Block (1941) administered large amounts of cholesterol dissolved in olive oil to two cretins without hypercholesteremia for periods of one and three weeks respectively. There was a negligible response in one and a slow and relatively small rise in the other. On the other hand Blaisdell and Chandler (1927) reported marked increases in dogs to which large quantities of cholesterol were administered in capsules for 38 to 119 days, and recently Steiner and Doman-

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ski (1941) obtained the same result in human patients and in dogs after the continued ingestion of 100 gm. egg yolk powder per day for periods of 4 to 34 weeks. In human subjects the average maximal increase was 101 mg. per 100 cc. serum and in dogs it was 203 mg. Because of Corwin's (1938) earlier finding that marked hypercholesteremia was produced in dogs by the feeding of phosphatides from the adrenal gland, Steiner and Domanski were inclined to ascribe their results to the phosphatides of the egg yolk rather than to the cholesterol, but later unpublished work induced Steiner[3] to doubt this explanation.

In a previous report, a definite hypercholestermia was observed in only one of 7 thyroidectomized monkeys. It seemed possible that a latent disturbance in cholesterol metabolism, such as occurs in man, might be present in these monkeys, and that hypercholesteremia did not appear because of the low level of cholesterol in the diet which they had received. The marked effect of fasting in reducing the hypercholesteremia of thyroidectomized dogs, reported by Entenman, Chaikoff, and Reichert (1942) after the experiments described here had been completed, is in accord with this interpretation. To test the hypothesis that the low cholesterol intake was responsible for the absence of hypercholestermeia, cholesterol was administered to the monkeys in the form of eggs and egg yolks. Later, after we had become acquainted with the findings of Steiner and Domanski (1941), the effect of cholesterol emulsions and solutions in oil and of egg phosphatides on the serum cholesterol concentration was studied.

EXPERIMENTAL

The experimental procedures used are described in the preceding paper (Jailer, *et al.*, 1944). Three monkeys were completely thyroidectomized, and four carried fragments of thyroid tissue. Total and free cholesterol concentrations were determined in the blood serum by a modification (Sperry, 1938; Macy, 1942) of the method of Schoenheimer and Sperry (1934). It should be stressed that continuous records were kept over long periods of time in the hypothyroid monkeys as well as in the control normal and spayed monkeys.

Single Administration of Eggs or Egg Yolks. Food was withdrawn from the cages at 2:00 P.M. and no further food was given. At 9:00 A.M. of the following day a sample of blood was taken and immediately thereafter several hard-boiled eggs were placed in the cage and left there until 11:00 A.M. when the uneaten portions were withdrawn. Blood samples were taken for analysis at intervals of 2 hours throughout the day and at 9:00 A.M. on the following morning. No food was given the monkeys during this time except that in two instances (cf. table 1), where only one egg was eaten during the first 2 hour period,

[3] Personal communication from Dr. A. Steiner.

additional eggs were offered at 5:00 P.M. and left in the cage during the night. In other experiments egg yolks were emulsified in 30 cc. of milk per four yolks and given by stomach tube with the aid of a 50 cc. syringe at the start of the experiment. An equal volume of water was flushed through the syringe and tube after each use.

Administration of Eggs or Egg Yolks for 4 days. The procedure was essentially the same as that described in the preceding section. The monkeys were allowed to eat as many eggs as they desired during the 4 days but in addition were offered only oranges and lettuce. In the experiments with egg yolks (cf. table 2) the yolks were emulsified in milk and given each morning by stomach tube. Blood samples were taken daily or every second day.

Administration of a Cholesterol Emulsion. Because of the possibility (Corwin, 1938) that a rise in serum cholesterol concentration following egg feeding is not caused by the cholesterol of the eggs, it was desirable to administer cholesterol in some other form in approximately the same quantity. Analysis of five egg yolks gave an average of 1.3 per cent cholesterol, or about 800 mg. in the four yolks administered per monkey per day in the preceding experiment. Hence an emulsion of cholesterol was prepared by beating 3.4 gm. cholesterol, 34.0 gm. of a cooking fat (Primex), and 1.36 gm. of glyceryl monstearate[4] in a beaker on the steam bath until a clear solution had been obtained. To this was added dropwise with mechanical stirring a solution of 0.68 gm. sodium borate and 6.8 gm. sodium alkyl sulfate[4] in 34 cc. water. The flask containing the latter solution was washed with two 17 cc. portions of water and these were added to the emulsion while stirring and heating were continued.

Approximately one-fourth of the stable, creamy emulsion was administered by stomach tube to each of 4 monkeys which had fasted overnight as in the preceding experiments. A fresh emulsion was prepared and given on each of 4 successive days, the whole procedure being the same as that employed with the egg yolks. The experiment was marred by vomiting, as indicated in table 3, probably induced by an irritating action of one of the detergents used in stabilizing the emulsion.

Administration of Cholesterol Dissolved in Oil. Because of the vomiting in the preceding experiment a solution of cholesterol in oil was administered to the same 4 monkeys. On the first day of the experiment each monkey was given 850 mg. of cholesterol in 12 gm. of vegetable (Puritan) oil. This proportion exceeded the solubility of cholesterol and there was some loss of crystallized cholesterol on the syringe and stomach tube. Hence, on the following 3 days the amount was increased to 900 mg. cholesterol to allow for wastage and the mixture was warmed to dissolve the cholesterol just before adminis-

[4] These products were supplied by the Procter and Gamble Company, Chemical Division, Ivorydale, Ohio.

istration. Some of the oil adhering to the syringe and stomach tube was carried into the stomach by a water washing and it was estimated that each monkey received at least 800 mg. of cholesterol, i.e. the approximate amount present in four egg yolks.

In a control experiment on the same monkeys the oil was administered in the same way without cholesterol.

Administration of Egg Phosphatides. The crude phosphatides were prepared from egg yolk powder by extraction with hot alcohol, evaporation to dryness in vacuo, extraction of the residue with ether, and precipitation with acetone. The product was purified by a second precipitation and given at the rate of 5 gm. per monkey per day. The

TABLE 1. INFLUENCE OF A SINGLE FEEDING OF EGGS OR EGG YOLKS ON THE CHOLESTEROL CONCENTRATION OF THE BLOOD SERUM

Monkey No.	Status[1]	Number of eggs or egg yolks (y)[2]	Total cholesterol					
			Control mg. %	2 hr.[3] mg. %	4 hr.[3] mg. %	6 hr.[3] mg. %	8 hr.[3] mg. %	24 hr.[3] mg. %
605	Nor.	3[4]	153	141	144	138	134	160
605	Nor.	4 (y)	149	161	153	149		170
609	Nor.	2	166	172	158	143	146	152
593	Spa.	4 (y)	122	119	119	115		128
614	Spa.	2	158	155	158	143	144	161
627	Thy.	2[5]	136	132	129	128	129	140
595	Spa-Thy.	4 (y)	151	153	156	156		165
613	Spa-Thy. Inc.	5	307	301	325	298	311	275
613	Spa-Thy. Inc.	4 (y)	259	270	262	250		259

[1] Nor. = normal; Spa. = spayed; Thy. = thyroidectomized. Thy-Inc. = with thyroid fragment.

[2] (y) = egg yolks.

[3] Time after feeding eggs or egg yolk administration. Uneaten eggs were removed at the end of the second hour.

[4] One egg eaten during first two hours. Two eggs eaten between 5:00 P.M. and 9:00 A.M. the next day.

[5] One egg eaten during first two hours. One egg eaten between 5:00 P.M. and 9:00 A.M. the next day.

phosphatides were prepared for administration by grinding in a mortar with a small amount of water to a thick paste which was then diluted to a concentration of 5 gm. in 50 cc. and administered by a stomach tube as in the preceding experiments. A fresh emulsion sufficient for 4 monkeys was prepared each day of the 4 day experiment.

DISCUSSION

It is clear from the results presented in tables 1 and 2 that a single feeding of eggs or egg yolks had no significant effect on the concentration of cholesterol in the blood serum either in normal, spayed, or hypothyroid monkeys during the following 24 hours. In only one instance (monkey 605, first experiment, table 2) did a large increase apparently occur, but the control value in this experiment is open to some question since it is considerably below the level usually

found in this animal. It may be objected that the number of eggs eaten in some of the experiments (only one during the first 2 hour period in two instances) was too small to cause an effect; but the cholesterol in a single egg is equivalent roughly to 70 mg. per 100 cc. of serum in monkeys of the size studied. The six experiments (table 1) in which three or more eggs were consumed with no apparent effect on the serum cholesterol concentration during the following 8 hours are considered adequate to demonstrate the absence of a rise during absorption; these together with the six experiments (table 2) in which there was no significant increase 24 hours after the ingestion

TABLE 2. INFLUENCE OF CONTINUED FEEDING WITH EGGS OR EGG YOLKS FOR FOUR DAYS ON THE CHOLESTEROL CONCENTRATION OF THE BLOOD SERUM

Monkey No.	Status	Eggs or egg yolks (y)[1]	Total cholesterol						
			Control mg. %	24 hr.[2] mg. %	48 hr.[2] mg. %	72 hr.[2] mg. %	96 hr.[2] mg. %	Maximum[3] mg. %	Maximal increase %
605	Nor.	3, 3, 2, 1	138	199	222	257	268	268 (4)	94.2
605	Nor.	4, 4, 4, 4 (y)	198	196	227	242	243	243 (4)	22.7
653	Nor.	13	155[4]		213		286	286 (4)	84.5
593	Spa.	4, 4, 4, 0 (y)	124[4]	133	191	224	224	224 (3)	80.6
614	Spa.	11	211[4]		229		283	283 (4)	34.1
614	Spa.	3, 3, 2, 1	150	162	186		231	231 (4)	54.0
627	Thy.	12	180[4]		229		235	235 (4)	30.6
627	Thy.	2, 2, 4, 3	150	165	210	198	200	210 (2)	40.0
595	Spa-Thy.	4, 4, 4[5], 4 (y)	150	136	147	209	207	221 (5)	47.3
613	Spa-Thy. Inc.	20	308[4]		436		526	526 (4)	70.8
613	Spa-Thy. Inc.	4, 3, 2, 2	242	273	315	339	384	384 (4)	58.7
613	Spa-Thy. Inc.	4, 4, 4, 4 (y)	252	245	297	362	361	448 (8)	77.8

[1] The figures represent in order the number of eggs eaten, or egg yolks given, on each of four consecutive days. A single figure is the total number of eggs eaten in four days. (y) indicates yolks given by stomach tube.
[2] Time after start of egg administration.
[3] Numbers in parenthesis represent the day after start of egg administration.
[4] Control value taken on day preceding the start of the experiment.
[5] Vomited.

of three or more eggs show that there is no post-absorptive response during this period (with the possible exception noted above).

However, when the administration of eggs was continued a definite rise was usually evident 48 hours after the first feeding (table 2) and in most cases the concentration of cholesterol continued to increase, reaching a maximum on the day following the last feeding, after which it fell abruptly. There was no difference in degree of response among normal, spayed, and hypothyroid monkeys. By far the largest increase in absolute amount was observed in monkey 613, the only one of the animals to show a definite hypercholesteremia (Jailer, *et al.*, 1944); but on a percentage basis the rise was no greater than that found in normal monkeys. The only deviation from the normal pattern was the continued rise for several days after egg yolk administration in one experiment on thyroidectomized monkey 613.

The foregoing results show that in the monkey, as in the other animal species which have been studied, no rise in the cholesterol concentration of the serum takes place during absorption; the animal organism is able to deposit in the tissues or to catabolize a large quan-

tity of cholesterol as rapidly as it can be absorbed. The thyroidectomized monkey apparently retains this ability unimpaired.

The marked increase in serum cholesterol which followed continued egg administration may be explained most readily by the assumption that the mechanism for disposal of cholesterol was unable to handle all of the excessive amounts ingested, with the result that a portion piled up in the blood. Against this interpretation is the well-established fact that the liver is able to accumulate large quantities

TABLE 3. INFLUENCE OF AN EMULSION OF CHOLESTEROL, A SOLUTION OF CHOLESTEROL IN OIL, AND OF EGG PHOSPHATIDES ON THE CHOLESTEROL CONCENTRATION OF THE BLOOD SERUM

Monkey No.	Status	Substance given	Total cholesterol[1]				
			Control mg. %	24 hr. mg. %	48 hr. mg. %	72 hr. mg. %	96 hr. mg. %
605	Nor.	Emulsion	161[v]	155	163	135	151
593	Spa.	Emulsion	134[v]	119	101	94[v]	90
595	Spa-Thy.	Emulsion	133[v]	130[v]	130[v]	129[v]	132
613	Spa-Thy. Inc.	Emulsion	250	250	229[v]	232[v]	205
605	Nor.	Solution	140	145	156	149	153
593	Spa.	Solution	98	111	131	148	155
595	Spa-Thy.	Solution	105	116	145	151	149
613	Spa-Thy. Inc.	Solution	194	202	222	232	241
605	Nor.	Oil alone	132	130	137	138	138
593	Spa.	Oil alone	118	115	126	143	137
595	Spa-Thy.	Oil alone	112	114	130	121	112
613	Spa-Thy. Inc.	Oil alone	188	189	223	218	212
605	Nor.	Phosphatides	131	138	117	112	121
605	Nor.	Phosphatides	136	138	141	145	145
593	Spa.	Phosphatides	112	109	97	99	114
593	Spa.	Phosphatides	112	110	107	107	108
595	Spa-Thy.	Phosphatides	156	125	117	108	105
595	Spa-Thy.	Phosphatides	108	112	108	104	110
613	Spa-Thy. Inc.	Phosphatides	227	216	181	189	203
613	Spa-Thy. Inc.	Phosphatides	235	215	205	222	197

[1] [v] indicates that vomiting occurred, usually 3 to 4 hours after the emulsion was given.

of cholesterol. It is unlikely that the capacity for storage in the liver and perhaps in other tissues was saturated by the quantities of cholesterol administered.

The finding of Corwin (1938) that administration of phosphatides to dogs causes hypercholesteremia through an unknown mechanism suggests the alternative explanation that the increase in serum cholesterol which followed continued egg administration was not a direct result of the egg cholesterol but a secondary response to the egg phosphatides. To test this hypothesis approximately the quantity of cholesterol present in four eggs was administered in the form of an emulsion and of a solution in oil during 4 consecutive days in experiments identical with those in which four egg yolks per day were given. No rise in serum cholesterol followed the administration of the cholesterol emulsion; in fact there was a decrease in two of the four experiments (table 3). The result is open to some question because of

vomiting which frequently occurred about 3 or 4 hours after administration. However, monkey 605 retained the emulsion on 3 consecutive days and monkeys 593 and 613 on 2 consecutive days without effect on the cholesterol concentration. A rise in serum cholesterol almost always occurred after 2 days of egg feeding.

After administration of cholesterol in oil a rise averaging 34.4 per cent at the maximum, was found (table 3). This finding is not entirely conclusive. A consideration of the continuous records shows that a decrease below the usual base line had occurred during the week intervening between the emulsion and solution in oil experiments, and that the control values from which the increases were calculated were depressed below the level which these monkeys ordinarily showed. For instance monkey 593, in which the largest rise occurred, maintained a level of about 125 mg. per 100 cc. over long periods of time. The control value of 98 mg. may have represented some delayed effect of the emulsion and the increase may have represented in part a spontaneous return toward the normal cholestrol concentration for this monkey. Furthermore, an average rise about half as large occurred in the control experiment in which the oil was administered with no cholesterol.

No increase in the cholesterol concentration of the serum followed the administration of egg phosphatides. On the contrary, there was a trend toward lower values in some of the experiments, of which the most marked example is the first on monkey 595. This particular result must be interpreted, however, in the light of a spontaneous, sharp rise for an unknown reason from the usual base line of about 125 to the control value of 156 mg. per 100 cc. The complete absence of hypercholesteremia after phosphatide administration is not necessarily in disagreement with Corwin's (1938) results because the species studied were different and the phosphatides were of different origin. It is unlikely that the time was too short in our experiments since Corwin obtained a maximal response in 1 week in some of his studies. The amount of phosphatide administered was about the same in proportion to body weight in the two investigations.

Limitations of space do not allow a detailed presentation of the data on free cholesterol which was determined throughout this investigation; but some of the findings are of sufficient interest to merit comment. It has been shown that in man the proportion between the cholesterol fractions is maintained within a narrow range of variation under all physiological and pathological conditions investigated, except in most types of liver disease and in acute infections; the ratio of combined to free cholesterol (C/F) is a "biological constant" in the human being (Sperry, 1936). Although the great majority of the C/F values obtained in monkeys in this series of investigations was within the same range of variation found in human subjects (2.3 to 3.1), fairly wide deviations were encountered. For instance in Monkey

593 C/F was usually below 2.0, a value which is definitely abnormal in a human being. Most of the monkeys studied showed occasional decreases in C/F to 2.0 or less, unassociated with any recognized physiological or pathological change. Characteristic examples are shown in figures 1 and 2. Since these deviations usually followed fairly smooth curves, it is unlikely that they represented experimental errors, and although the number of animals studied is too small to jus-

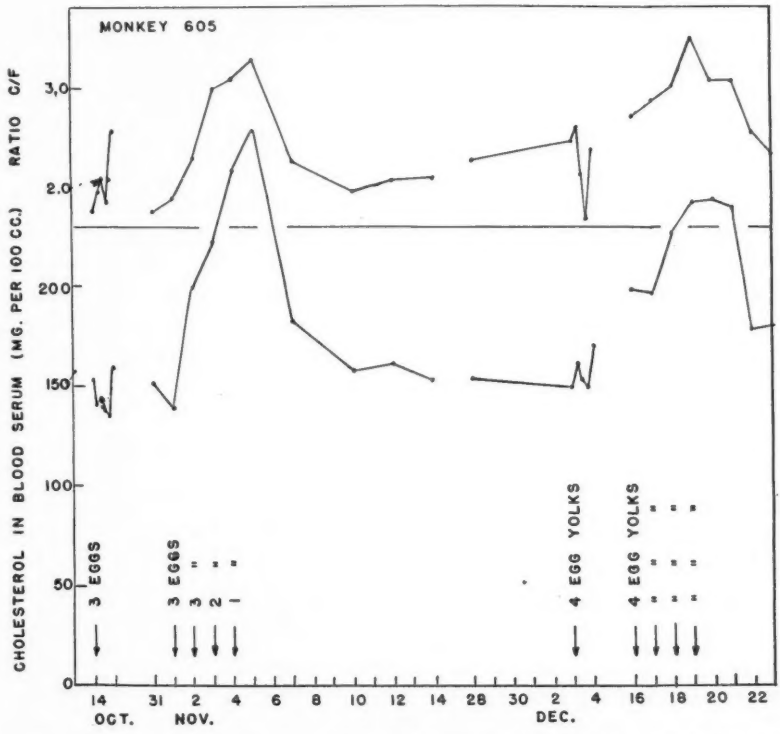

FIG. 1. Effect of eggs or egg yolks on the ratio of combined to free cholesterol (C/F) and on the total cholesterol concentration of the blood serum in a normal monkey. The curves shown are taken from a continuous record.

tify a definite conclusion, it appears that the monkey does not maintain the proportion between the cholesterol fractions with the rigidity which is characteristic of the human being. Further evidence in accord with this interpretation is the finding that a rise in C/F occurred consistently along with the rise in total cholesterol which followed the administration of eggs. Frequently, as in the instances shown (Figs. 1 and 2), C/F rose considerably above the upper limit of normal in man. Values above the normal range have almost never been encoun-

tered in human beings (Sperry, 1936; Hodges, *et al.*, 1943) nor were they seen in the monkeys except under these conditions. A rise in C/F along with a rise in total cholesterol shows, of course, that combined cholesterol increased proportionally more than did free cholesterol. However, free cholesterol also rose in most of the experiments.

Another indication of the lability of C/F in monkeys was a considerable fluctuation in this ratio observed in a large proportion of the experiments following the administration of various substances even

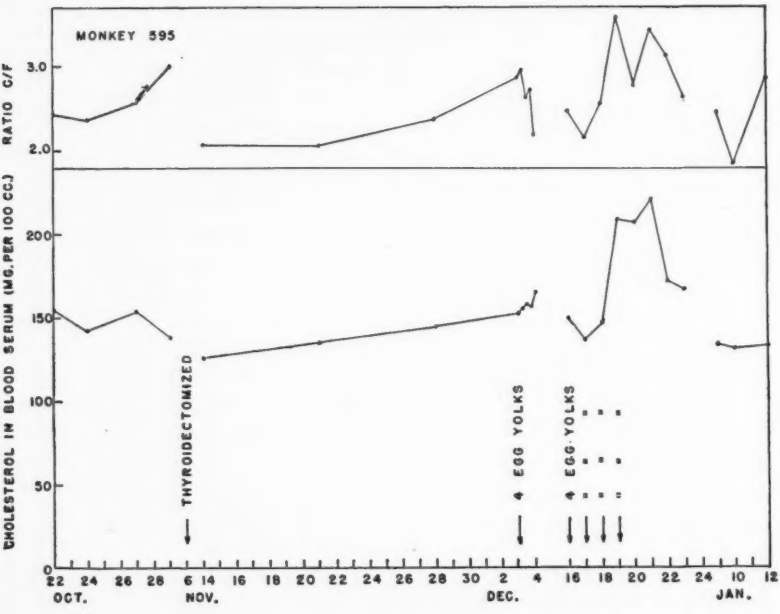

FIG. 2. Effect of egg yolks on the ratio of combined to free cholesterol (C/F) and on the total cholesterol concentration of the blood serum in a thyroidectomized monkey.

though the total cholesterol concentration was not significantly affected. Examples are shown in figures 1 and 2 in the experiments in which cholesterol was determined at 2-hour intervals following a single administration of eggs. Considerable variations in C/F were found in most of the experiments after the administration of cholesterol emulsions or solutions, of oil, and of egg phosphatides. It is of interest that C/F rose considerably in three of the emulsion experiments even though total cholesterol was not affected; the curves were the same in general shape and magnitude of change as those obtained after egg feeding.

No evidence for a latent disturbance in cholesterol metabolism in thyroidectomized monkeys was obtained in this investigation; in none of the experiments did we observe any significant difference in response among thyroidectomized, normal and spayed animals, with the possible exception of the continued rise in serum cholesterol after egg administration had stopped in one experiment on Monkey 613. Entenman, Chaikoff, and Reichert (1942), in the only other study which has come to our attention of the influence of diet on the cholesterol concentration of the blood in thyroidectomized animals, found a marked effect of variations in dietary intake; but the two investigations can not be directly compared because the monkey and the dog, which they studied, respond quite differently to thyroidectomy, and because the variations in diet were different: fasting vs. addition of various cholesterol-containing preparations to the diet. Their conclusion that changes in total cholesterol under their conditions were predominantly in the combined cholesterol fraction is of interest in view of our finding that combined cholesterol rises more than free cholesterol following egg feeding. Unfortunately, their analysis of whole blood instead of serum makes it impossible to evaluate changes in free cholesterol which was found to increase along with the combined fraction in our experiments.

SUMMARY

The concentration of cholesterol in the blood serum did not change significantly in normal, spayed, or hypothyroid monkeys during the 8 hours following the ingestion of several eggs, nor was there any postabsorptive change 24 hours later. However, when egg feeding was continued for 4 days, a definite rise was usually evident on the second day and a maximum, averaging more than 50 per cent above the starting value, was reached usually on the day after egg feeding was stopped. Both combined and free cholesterol increased, the combined fraction proportionately more than the free.

No rise in cholesterol concentration followed the administration for 4 days of an amount of cholesterol, approximately equivalent to that ingested in the eggs, in the form of an emulsion. This result was not entirely conclusive because of some vomiting.

A slight rise in cholesterol concentration, somewhat smaller than that which followed egg feeding, occurred after the same amount of cholesterol had been given for 4 days in oil solution. About half as large an increase was found when the same amount of oil was given under the same conditions to the same monkeys.

Administration of egg phosphatides for 4 days did not cause any increase in serum cholesterol concentration.

Evidence was obtained indicating that the proportion between the

cholesterol fractions of the blood serum is not controlled by the monkey with the rigidity which characterizes the human being.

No evidence for a latent disturbance in cholesterol metabolism in hypothyroid monkeys was obtained; in none of the experiments, with one possible exception, did thyroidectomized animals respond in a manner different from that manifested by normal and spayed monkeys.

REFERENCES

BLAISDELL, F. E., AND L. R. CHANDLER: *Am. J. M. Sc.* 174: 492. 1927.
CORWIN, W. C.: *Arch. Path.* 26: 456. 1938.
ENTENMAN, C., I. L. CHAIKOFF, AND F. L. REICHERT: *Endocrinology* 30: 794. 1942.
FRÖHLING, W.: *Arch. f. Verdauungskr.* 59: 205. 1936.
HODGES, R. G., W. M. SPERRY, AND D. H. ANDERSEN: *Am. J. Dis. Child.* 65: 858. 1943.
JAILER, JOSEPH W., W. M. SPERRY, E. T. ENGLE, AND GEORGE SMELSER: (In Press).
MACY, I. G.: Nutrition and Chemical Growth in Childhood, Springfield. 1: 331. 1942.
MJASSNIKOW, A.: *Ztschr. f. klin. Med.* 103: 767. 1926.
MJASSNIKOW, A., AND B. ILJINSKY: *Ztschr. f. d. ges. exper. Med.* 53: 100. 1926–27.
SCHOENHEIMER, R., AND W. M. SPERRY: *J. Biol. Chem.* 106: 745. 1934.
SPERRY, W. M.: *J. Biol. Chem.* 114: 125. 1936.
SPERRY, W. M.: *Am. J. Clin. Path.*, 2: 91. 1938.
SSOKOLOFF, N. A.: *Arch. path. Anat.* 245: 203. 1923.
STEINER, A., AND B. DOMANSKI: *Am. J. M. Sc.* 201: 820. 1941.
WILKINS, L., W. FLEISCHMANN, AND W. BLOCK: *J. Clin. Endocrinol.* 1: 3. 1941.

THE EXTRACTION OF CORTIN-LIKE SUBSTANCES FROM HUMAN POST-OPERATIVE URINE[1]

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THE CORTIN-LIKE PROPERTIES of extracts of urine from various sources have been demonstrated by several investigators. Perla and Marmorston-Gottesman (1931) reported that a benzene extract of the urine of young men and women was able to increase the resistance of adrenalectomized rats to histamine poisoning. It was estimated that 1 liter of urine contained an amount of protective substances equivalent to that obtained from approximately 225 gm. of beef adrenal by the method of Hartman (1930). More convincing proof of the presence of substances with cortin-like activity was provided by the experiments of Grollman and Firor (1932) which showed that benzene extracts of the urine of men and women were effective in prolonging the survival period of the adrenalectomized rat. The extract of 1 liter of urine was equivalent in activity to 0.5 gm. of adrenal tissue (Grollman and Firor, 1933). Weil and Browne (1939a) showed that the urine of some normal individuals contained a substance, extractable with ethylene dichloride, which increased the resistance of adrenalectomized rats to cold. This has been confirmed by Dorfman, Horwitt and Fish (1942), who, in addition, have demonstrated that the ethylene dichloride extracts of the urine of normal men exhibit other cortin-like properties. These extracts were effective in maintaining the life of the adrenalectomized rat (Dorfman and Horwitt, 1943), in inducing the deposition of glycogen in the liver of the fasted adrenalectomized rat (Horwitt and Dorfman, 1943) and in preventing water intoxication in the adrenalectomized rat (Schiller and Dorfman, 1943).

The blood and urine of patients suffering from a variety of conditions have been shown to contain an increased amount of cortin-like substances. Extracts of the blood and urine of patients with Cushing's syndrome (Anderson and Haymaker, 1937, 1938a, b) were effective in prolonging the life of adrenalectomized rats. Extracts which were active in the Selye-Schenker cold exposure test have been prepared from the urine of patients with Cushing's syndrome, hypertension,

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acute and chronic infections and of patients who had been burned or exposed to surgical procedures (Weil and Browne, 1939a, b, 1940, 1944).

Selye and Dosne (1940) have reported that the urine of heifers, mares and pregnant cows contains substances which maintain the adrenalectomized rat exposed to cold.

The present report deals with a detailed description of the method of preparation of extracts with cortin-like activity from human post-operative urine and of the metabolism of adrenal cortical extract administered to man. A preliminary account has been published elsewhere (Venning, Hoffman and Browne, 1943).[3]

EXPERIMENTAL PROCEDURE

BIOLOGICAL ASSAY

Cold Exposure Test. This method of assay depends on the preservation of life in adrenalectomized immature rats under the stress of exposure to low environmental temperatures. The procedure of Selye and Schenker (1938), slightly modified, has been used in this investigation.

Hooded rats of either sex weighing between 40 and 60 grams are bilaterally adrenalectomized under ether anaesthesia by the usual dorsal approach. In the present experiments male rats have been used almost exclusively. Although the amounts of adrenal cortical extract required to maintain adrenalectomized male or female rats exposed to cold are not significantly different, the mean survival time of the untreated males and females, under the conditions of the assay, is not the same. Therefore males and females should not be used in the same assay. Tyslowitz and Astwood (1942) found that, with colonic temperature as a criterion, adrenalectomized female rats were more resistant to cold than adrenalectomized males.

Sixteen to 24 hours after adrenalectomy, food (Purina fox chow) and water are withdrawn, and those animals that have not recovered from the operation are discarded. Three groups of 15 animals each are separated from the remainder. One group serves as a control and each rat of this group receives 1 cc. of water by gavage. It has been found necessary to use a control group with each assay as considerable variation in sensitivity to cold has been observed in different groups of untreated animals even when the conditions of the test are strictly adhered to. The other 2 serve as test groups (any number of test groups can be used with one control). In the original technique of Selye and Schenker, groups of 9 rats were used.

Each rat in the test group is given an equal amount of the extract to be assayed by stomach tube. The extract is so prepared that the required dose is usually given in 1 cc. of water or aqueous ethanol (10%). The oral route has been used in preference to the parenteral as many of the extracts to be tested were found to be toxic when administered by the latter method.

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[3] Progress reports have been made to the National Research Council of Canada (1942–1944) and to the Conference on Metabolic Aspects of Convalescence Including Bone and Wound Healing (1943–1944) held under the auspices of the Josiah Macy, Jr., Foundation.

Selye and Schenker administered the extract in 3 doses, one immediately before exposure to cold, and the others at 3 hour intervals during exposure. In the present procedure the material has been given in one administration as it is doubtful that extracts administered during the period when body temperature is falling would have an effect on survival (Roos, 1943). The animals are inspected one-half hour following the administration of the extract and any that appear to have been damaged by the gavage procedure are discarded. They are then placed in a galvanized iron box ($15 \times 12 \times 7\frac{1}{2}$ inches) which is divided into 3 compartments, one for each group of rats. The box is provided with a cover made of one-quarter inch mesh wire. This procedure was found to be more satisfactory than placing each animal in an individual compartment. The box is then placed in a refrigerator or cold room which is maintained between 0° and −4° C. If a refrigerator is used it is desirable to have a fan circulating the air over the cold coils in order to equalize the temperature. Because there are differences in sensitivity to cold in different strains of rats the temperature should be so adjusted that the control animals begin to die within 6 to 8 hours following exposure. The box is removed from the refrigerator and the animals inspected at room temperature as rapidly as possible, at the end of 3 hours and at 30 to 45 minute intervals thereafter, depending on the rate at which they die.

The condition of the animals is categorized as follows: (a) Active (animals run around). (b) Inactive (animals are sluggish but will right themselves when placed on their backs). (c) Comatose (animals do not move but are still breathing). (d) Dead.

The test is considered to be positive when at the time that at least 10 of the control rats are either comatose or dead, at least 10 of the treated animals are active or inactive. *A Cold Unit* (*C.U.*), as used in this communication, may therefore be defined as the minimum amount of an extract or substance which when administered to a group of 15 adrenalectomized immature rats one-half hour prior to their exposure to cold, will maintain at least two-thirds of them in the active or inactive state at a time when at least two-thirds of the controls are either comatose or dead.

Dorfman, Horwitt and Fish (1942) and Vogt (1943), in applying the cold exposure test, use the increase in mean survival time of the treated over the untreated adrenalectomized rats as the criterion of a positive response. Recently Roos (1943) has described a similar assay procedure for adrenal cortical extracts which is concerned with the maintenance of the body temperature of adrenalectomized rats exposed to an environmental temperature of 1° C. In the opinion of this author the determination of the fall in colonic temperature is a more satisfactory criterion than coma or death, as a smaller group of animals is required to yield significant results. Although a direct comparison of the 2 methods has not been made it appears that they have the same order of sensitivity.

Zarrow (1942) demonstrated that desoxycorticosterone acetate at dose levels of 250 and 500 μg, and progesterone at 500 μg, were effective in prolonging the life of adrenalectomized mice subjected to a low temperature. Weil (1941) and the present authors found that the mouse, due to its varying sensitivity, was an unsatisfactory test animal in the cold exposure test as outlined above.

The activity of various adrenal cortical extracts and crystalline steroids is shown in table 1. Desoxycorticosterone acetate and progesterone were administered subcutaneously in 0.2 cc. of sesame oil while the control rats received an equal amount of oil. As the amount of adrenocortical substance required to protect the adrenalectomized rat against the lethal action of cold may change, due to the varying sensitivity of the test animals, it is important that the cold unit equivalent of a reference standard be determined at regular intervals.

Survival-Growth Test. Male rats of a hooded strain, weighing between 38 and 45 grams, were bilaterally adrenalectomized (it was found necessary to strip the capsule from the upper pole of the kidney). Only those rats which, 48 hours after the operation, showed no gain in weight were used. The ad-

TABLE 1. ACTIVITY OF ADRENAL CORTICAL EXTRACTS AND STEROIDS IN THE COLD EXPOSURE TEST

Preparation	Route of administration	1 cold unit	Author
Adrenal Cortical Extract (Connaught Laboratories)	Oral	0.05 cc.	Present authors
Adrenal Cortical Extract (Upjohn)	Oral	0.05 cc.	Present authors
Adrenal Cortical Extract (Eschatin, Parke, Davis & Co.)	Oral	0.066 cc.	Present authors
Corticosterone	Oral	10 μg	Present authors
Desoxycorticosterone Acetate	Subcutaneous	1.5–2 mg.	Present authors
Progesterone	Subcutaneous	negative at 2 mg.	Present authors
Corticosterone	—	10 μg	Kendall (1941)
17-hydroxy-dehydrocorticosterone	—	18–20 μg	Kendall (1941)
Amorphous Fraction	—	13 μg	Kendall (1941)

renal cortical and urinary extracts were administered subcutaneously in 0.5 cc. of water twice daily for 12 days. The survival period and weight changes of the treated rats were compared with those of untreated adrenalectomized animals (table 3). During the course of the test the animals were maintained on Purina fox chow and water.

Test for Determining the Effect on the Deposition of Liver Glycogen. The capacity of the urinary extracts to influence the deposition of glycogen in the liver of the fasted adrenalectomized rat was measured by the method of Reinecke and Kendall (1942).

Hooded male rats, weighing 120 to 160 grams, were adrenalectomized and maintained on Purina fox chow and a 1% solution of sodium chloride. Seventy-two hours after adrenalectomy food was withdrawn and 24 hours later the rats were given hourly administrations of the extract either subcutaneously or orally. For 7 hours the required dose being in solution in 0.5 to 1 cc. of 10% ethanol. Between 1½ and 2 hours after the last administration the animals were anaesthetized with sodium amytal given intraperitoneally (9 mg. per 100 gm. body weight). The livers were quickly dissected out and immersed in 10 cc. of a hot 30% solution of potassium hydroxide. The glycogen content was measured by the method of Good, Kramer and Somogyi (1933). The glycogen is expressed as glucose and is recorded as mg. of glucose per 100 gm. of body weight.

EXTRACTION AND PURIFICATION OF THE CORTIN-LIKE SUBSTANCES IN POST-OPERATIVE URINE

The urine is collected without preservative for the first 7 post-operative days from patients who have undergone major operations.[4] The daily unhydrolyzed specimens are pooled and extracted within 48 hours of collection with ethylene dichloride. Chloroform may be used as a preservative if the urine has to be stored. Three extractions are made with 150, 150 and 100 cc. of ethylene dichloride for each liter of urine. Emulsions, which frequently form can be broken by centrifugation or by allowing the extract to stand over night. The combined ethylene dichloride extracts are evaporated almost to dryness under reduced pressure at a bath temperature not exceeding 50° C. This bath temperature is used throughout the subsequent fractionation procedure in order to prevent destruction of the active compounds. The residue (I) representing 1 liter of urine weighs 30 to 38 mg. at this stage. It is taken into solution in a small volume of ethanol and stored in the cold until 300 liters of urine have been extracted.

The volumes of solvents given in the subsequent procedures refer to the processing of a batch of 300 liters of urine and the weights of the residues are expressed in terms of 1 liter of urine.

The ethanol of the combined extracts is removed and the residue (I) is dissolved in 150 cc. of 70% ethanol. This solution is extracted with petroleum ether, B.P. 20–40° C. (3×50 cc.). The petroleum ether phase is back extracted with 70% ethanol which is added to the original solution. Extraction with petroleum ether removes 9–12 mg. of impurities (residue II), leaving 20–25 mg. (residue III) in the ethanol. The aqueous ethanolic solution is concentrated in vacuo to remove the ethanol and water is added to bring the volume to 150 cc. This aqueous solution is extracted with chloroform (10×70 cc.) and then is discarded. The combined chloroform extracts are chilled and extracted with cold 0.1 sodium hydroxide solution (3×60 cc.). This procedure is carried through quickly and each alkali washing is back extracted with 50 cc. of chloroform. If emulsions form they are easily broken by centrifugation. The extraction with alkali removes considerable amounts of impurities including the sulphonamide excretion products frequently present in this type of urine.

The chloroform extracts are combined and washed with water, usually 3 washings totalling 60 cc. of water are required, the water washings are back extracted with chloroform. The combined chloroform extracts are dried with anhydrous sodium sulphate and evaporated almost to dryness under reduced pressure. The last traces of chloroform are removed by a stream of nitrogen at room temperature. The weight of the residue (IV) at this stage is reduced to 9–12 mg. It is taken into solution with 120 cc. of benzene which is then extracted with water (10×100 cc.). Care must be taken to avoid emulsions. The benzene extract is dried over anhydrous sodium sulphate, evaporated and the residue stored in ethanol in the cold for further investigation. The benzene soluble material (residue V) weighs 6.5–8.5 mg.

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[4] We are indebted to the Royal Victoria Hospital, Montreal General Hospital, Hotel Dieu, Notre Dame Hospital and Ste. Anne's Military Hospital for providing the urine used in this study.

The combined water washings are saturated with sodium chloride and extracted with chloroform (7×100 cc.). The chloroform extracts are dried over anhydrous sodium sulphate, filtered and evaporated under reduced pressure. The residue (VI), which weighs 2.2–3.5 mg., is separated into ketonic and non-ketonic substances by means of Girard's reagent T as described by Reichstein (1936). To a solution of this residue in 10 cc. of methanol are added 1.2 gm. of Girard's reagent T and 0.6 cc. of glacial acetic acid. The mixture is allowed to stand at room temperature for 15 hours. It is then chilled and sufficient 1 N sodium carbonate solution is added to neutralize the acid. The methanol is evaporated in vacuo at 0° C. Water is added to bring the volume to 20 cc. and the solution is extracted with chloroform (7×20 cc.). The chloroform extracts are dried and evaporated in vacuo. The non-ketonic residue (VII) weighs 1.5–2.5 mg.

The ketones that reacted with Girard's reagent are divided into 3 fractions by adjusting the pH of the aqueous solution to 4, 2 and finally 0, and extracting with chloroform (7×20 cc.) after each acidification. The chloroform extracts, containing the free ketones, are washed free of acid with 15 cc. of a sodium carbonate–sodium bicarbonate solution of pH 9 and then washed with water (3×10 cc.). Each of these washings is back extracted with chloroform. The chloroform extracts are dried with anhydrous sodium sulphate and evaporated in vacuo. The distribution of the ketonic material is as follows: 0.06–0.13 mg. are obtained at pH 4; 0.033–0.06 mg. at pH 2; and 0.11–0.25 at pH 0.

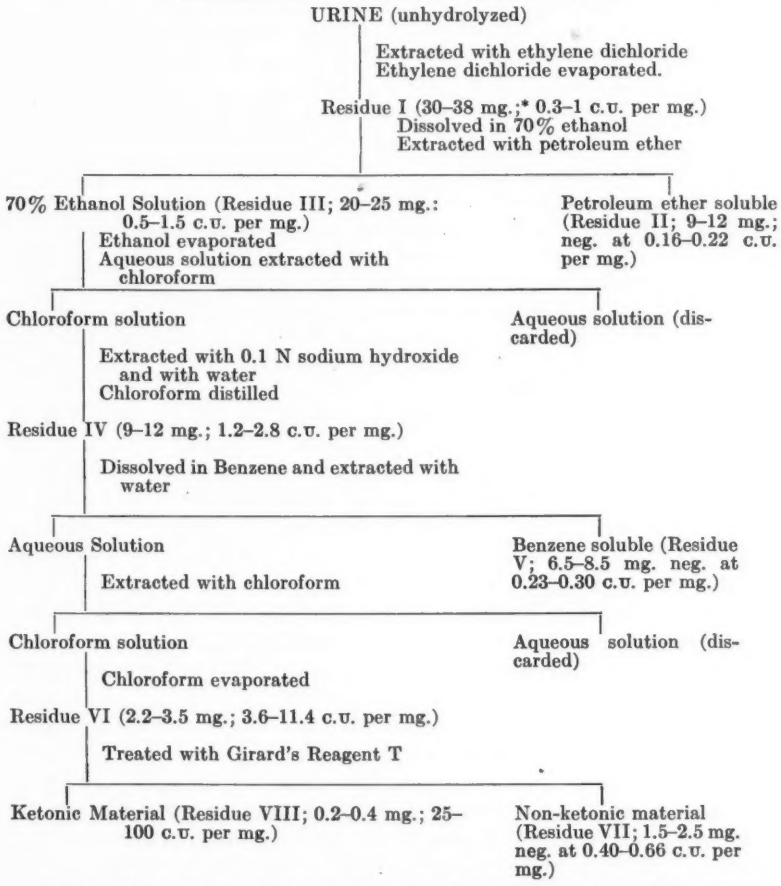
RESULTS

The observation of Weil and Browne (1939b, 1940, 1944) that ethylene dichloride extracts of post-operative urine are active in the cold exposure test was confirmed. In an effort to concentrate the active substances so that further physiological and chemical studies could be made, the urinary extracts were fractionated by distribution between various solvents as outlined in the flow sheet.

The activity of the fractions during the various stages of purification was determined by means of the Selye-Schenker cold exposure test and in many instances the capacity of the extracts to influence the deposition of glycogen in the liver of the fasted adrenalectomized rat was used as an additional criterion. The cold exposure test has distinct advantages over other methods of assay for this purpose as it is highly sensitive, rapid and easy to apply. In addition, it has the added advantage, as is apparent from table 1, that it is not specific for any particular fraction of the adrenal cortical steroids. Adrenal cortical extracts are very active in this test, 1 C.U. being contained in 0.05–0.066 cc. Corticosterone, 17-hydroxy-dehydrocorticosterone and the amorphous fraction of Kendall are active at a dose level of 10–20 μg. Desoxycorticosterone acetate, on the other hand, is much less active, 1 C.U. being equivalent to 1.5–2 mg. Progesterone is inactive at a dose level of 2 mg.

Different lots of pooled post-operative urine varied considerably in their activity. The material extracted with ethylene dichloride con-

FLOW SHEET OF FRACTIONATION PROCEDURE FOR PREPARATION OF EXTRACTS WITH CORTIN-LIKE ACTIVITY FROM POST-OPERATIVE URINE


URINE (unhydrolyzed)
Extracted with ethylene dichloride
Ethylene dichloride evaporated.
Residue I (30–38 mg.;* 0.3–1 c.u. per mg.)
Dissolved in 70% ethanol
Extracted with petroleum ether
70% Ethanol Solution (Residue III; 20–25 mg.: 0.5–1.5 c.u. per mg.)
Ethanol evaporated
Aqueous solution extracted with chloroform
Petroleum ether soluble (Residue II; 9–12 mg.; neg. at 0.16–0.22 c.u. per mg.)
Chloroform solution
Aqueous solution (discarded)
Extracted with 0.1 N sodium hydroxide and with water
Chloroform distilled
Residue IV (9–12 mg.; 1.2–2.8 c.u. per mg.)
Dissolved in Benzene and extracted with water
Aqueous Solution
Benzene soluble (Residue V; 6.5–8.5 mg. neg. at 0.23–0.30 c.u. per mg.)
Extracted with chloroform
Chloroform solution
Aqueous solution (discarded)
Chloroform evaporated
Residue VI (2.2–3.5 mg.; 3.6–11.4 c.u. per mg.)
Treated with Girard's Reagent T
Ketonic Material (Residue VIII; 0.2–0.4 mg.; 25–100 c.u. per mg.)
Non-ketonic material (Residue VII; 1.5–2.5 mg. neg. at 0.40–0.66 c.u. per mg.)

* Weights in mg. throughout represent the amounts of extractives per liter of urine.

tained 0.3–1 c.u. per mg. (residue I). The extract at this stage was frequently quite toxic to the adrenalectomized rat, due probably to large amounts of sulphonamide excretion products which are usually present in pooled post-operative urine. By partitioning the ethylene dichloride soluble material between 70% ethanol and petroleum ether considerable amounts of impurities were removed. The petroleum ether soluble material (residue II) was inactive at a dose level of 0.16–0.22 c.u. per mg. The biologically active substances remained in the aqueous ethanol phase and after removal of the ethanol they could be extracted with chloroform. The chloroform soluble material (residue III) contained 0.5–1.5 c.u. per mg.

It was found that a solution of the crude urinary extract in chloroform could be shaken with 0.1 N sodium hydroxide solution without any loss of activity. This procedure effected considerable purification. The residue (IV), obtained after removal of organic acids and phenols with alkali, now contained 1.2–2.8 C.U. per mg.

The next step in the purification process, which consisted of partitioning the material between benzene and water, still further concentrated the biologically active material. The active substances passed from benzene into water. Residue V, obtained from the benzene solution, was negative when assayed at 0.23–0.30 C.U. per mg., while the water soluble residue VI contained 3.6–11.4 C.U. per mg. An alcoholic solution of the water soluble residue appears to be quite stable as it can be stored in ethanol for several months at 0° C. without any detectable loss in activity. This fraction was found to reduce alkaline silver solution strongly and rapidly at room temperature. It was not precipitated by digitonin.

Approximately 20% of the activity of residue VI was lost when it was separated into ketonic and non-ketonic fractions. The non-ketonic material (residue VII) was inactive at a dose level of 0.40–0.66 C.U. per mg. The total ketonic fraction (residue VIII), on the other hand, was highly active, containing 25–100 C.U. per mg. The ketonic substances were found to be unstable and could not be stored in ethanol at 0° C. for more than a week without loss of activity.[5]

The effectiveness of various fractions of the urinary extract in influencing the deposition of glycogen in the liver of the fasted adrenalectomized rat was measured and compared with the response elicited by adrenal cortical extract (table 2).

In a series of 28 control rats the liver glycogen ranged from 0.5–1.2 mg. per 100 gm. of body weight, with an average value of 0.8 mg. Adrenal cortical extract at a dose level of 35 C.U. (1.75 cc.) caused an average deposition of 13.9 mg. of glycogen per 100 gm. of body weight in a group of 9 rats. When the dose was doubled the liver glycogen was increased to an average of 39.4 mg. in the same number of animals. The water soluble residues (VI) of the extracts of three different lots of post-operative urine were injected at a dose level of 70 C.U. to groups of 3 or 4 rats. The average amounts of glycogen deposited were 16.7, 14.5 and 13.8 mg. When the dose level of one of these extracts (L-3) was increased to 140 C.U., which in this instance represented the extract of 28 liters of urine, the average glycogen deposition (9.9 mg.) was actually lower than that deposited (13.8 mg.) by half the dose. With the higher dose toxic effects were observed and it has been our experience that when extracts are toxic the deposition

[5] From the acetylated ketonic residue there has been isolated by chromatographic analysis an unidentified crystalline substance melting at 234–236° C. This substance, which reduces alkaline silver solution at room temperature, is active in the cold exposure test.

of glycogen is interfered with. Oral administration reduces the toxicity. Thus when 140 C.U. of residue VI of L-4 were given orally toxic manifestations were not observed and the deposition of glycogen in 4 animals averaged 23.7 mg.

An extract of the total ketonic fraction given at a level of 84 C.U. caused an average deposition of 10.6 mg. of glycogen per 100 gm. of body weight. This extract was toxic which probably explains the poor response and the wide variations (3.3–24.6 mg.).

TABLE 2. EFFECT OF ADRENAL CORTICAL AND URINARY EXTRACTS ON DEPOSITION OF LIVER GLYCOGEN

Extract	Total dose		No. of rats	Liver glycogen per 100 gm. of body wt.		Comments
	Cold units	Urine equivalents in liters		Range	Average	
				mg.	mg.	
Controls			28	0.5– 1.2	0.8	
Adrenal cortical extract*	35 (1.75 cc.)		9	8.0–28.1	13.9	
Adrenal cortical extract*	70 (3.50 cc.)		9	32.3–51.9	39.4	
Water soluble Residue VI						
L-1	70	4.2	4	11.8–23.5	16.7	
L-2	70	22.4	3	12.1–18.7	14.5	
L-3	70	14.0	4	12.4–15.9	13.8	
	140	28.0	6	1.7–18.0	9.9	marked toxic symptoms
L-4	140†	14.0	4	12.2–29.5	23.7	
Benzene soluble Residue V	negative	7.0	2	1.4– 1.6	1.5	toxic symptoms
		14.0	2	1.4– 2.0	1.7	toxic symptoms
Ketonic Residue VIII	84‡	17.5	5	3.3–24.6	10.6	toxic symptoms
Non Ketonic Residue VII	negative	19.6	6	0.3– 0.5	0.4	toxic symptoms

Extracts are given subcutaneously in 7 hourly doses unless otherwise stated.
* Product of Connaught Laboratories.
† Extract given orally in 4 divided doses over a period of 7 hours.
‡ 4 administrations subcutaneously and 3 orally.

The benzene soluble residue (V) and the non-ketonic fraction were also tested although they failed to show any activity in the cold exposure test. Both extracts were ineffective in causing glycogen deposition. It should be noted, however, that these extracts were toxic.

The urinary cortin-like substances were found to increase the survival time of the adrenalectomized rat (table 3). Thus the daily subcutaneous administration of 40 C.U. of the water soluble fraction (residue VI) of the urinary extract to 20 adrenalectomized immature rats maintained 16 of them during the period (14 days) of the assay. Withdrawal of the extract resulted in the death of all 16 animals in an average time of 4.8 days. The average survival period of 33 untreated adrenalectomized rats was 4.9 days. It is to be noted that at 40 C.U.

TABLE 3. EFFECT OF URINARY CORTIN-LIKE SUBSTANCES ON SURVIVAL AND GROWTH OF ADRENALECTOMIZED RATS

Extract	Daily dose	No. of rats injected	Average gain in weight in 14 days	No. of rats surviving on 14th day	Average survival time after last injection
	Cold Units		gm.		days
Untreated controls		33		0*	
†Adrenal Cortical Extract	20	9	22.4	9	3.7
Water Soluble Extract (Residue VI)	40	20	8.9	16	4.8

* The average survival time was 4.9 days.
† Product of Connaught Laboratories.

the urinary extract was less effective in inducing gain in weight (8.9 gm.) than adrenal cortical extract at 20 c.u. (22.4 gm.).

To determine the metabolic fate of the biologically active adrenocortical steroids in man, varying amounts of adrenal cortical extracts (134–1200 cc.) which had been assayed in this laboratory by the cold exposure test were administered to 3 patients as shown in table 4. We are indebted to Drs. E. C. Reifenstein, L. W. Kinsell and Fuller Albright of the Massachusetts General Hospital for providing us with the urine of patients D and M'C. The ethylene dichloride extracts of aliquots of urine collected during the period of hormone therapy, and in the case of patient M for the following 2 days, were assayed by the

TABLE 4. THE RECOVERY OF CORTIN-LIKE SUBSTANCES FROM URINE FOLLOWING THE ADMINISTRATION OF ADRENAL CORTICAL EXTRACT

Patient	Adrenal cortical extract administered				Recovery of cortin-like substances in urine	
	Route of administration	Am't per day	Total am't administered		Cold units	Percentage of dose administered
		cc.	cc.	Cold Units		
*D—Male, 60 yrs. carcinoma of prostate with metastases.	intravenous	100	900[1]	16,500	2030	12.3
*M'C—Male, 25 yrs. fracture of right femur.	80 cc. intravenous 20 cc. subcutaneous	100	1200[2]	24,000	1680	7.0
M—Male, 40 yrs. 1st and 2nd degree burns	107 cc. intravenous 27 cc. subcutaneous	134	134[3]	2,680	280	10.4

[1] 600 cc. of adrenal cortical extract (Upjohn) and 300 cc. of Eschatin (Parke, Davis & Co.).
[2] Adrenal cortical extract (Upjohn).
[3] Adrenal cortical extract (Connaught).
* Urine provided through the courtesy of Drs. E. C. Reifenstein, L. W. Kinsell, and Fuller Albright of the Massachusetts General Hospital, Boston.

cold exposure test. Urine collected from each patient during a control period was negative when assayed at a level of 5–15 C.U. per day. On the assumption that all the cortin-like substances excreted during the treatment period was derived from the injected extract, from 7 to 12.3% of the biological activity of that extract was recovered from the urine.

DISCUSSION

The foregoing experiments have demonstrated that there are present in human post-operative urine substances which are able: (a) to protect adrenalectomized rats against the lethal action of low temperatures, (b) to sustain life and growth in the adrenalectomized rat and (c) to induce deposition of glycogen in the liver of the fasted adrenalectomized rat. The urine of normal men contains substances with the same biological properties but in smaller quantities. Thus post-operative urine contains 10–30 C.U. per liter whereas the urine of normal men is positive in the cold exposure test at a level of 1–3 C.U. per liter.

The method of purification which has been described permits on the average a 100 fold concentration of the active substances with a loss of approximately 25% of the activity present in the initial crude extracts of post-operative urine. Whether the active substances possess still other biological properties characteristic of the adrenocortical steroids has not yet been ascertained. In view of the relative specificity, however, of the properties that have already been shown to be characteristic of the post-operative urinary extract, in particular that of life maintenance and the effect on carbohydrate metabolism, it seems likely that the active urinary substances are metabolites of the adrenocortical steroids.

Although only incomplete data are available at present concerning the chemical properties of these substances, their behavior on partition between various solvents and the possession of a reactive carbonyl group relates them chemically to the known physiologically active steroids of the adrenal cortex. If the urinary material represents a transformation product of adrenocortical secretion one might expect it to be a mixture rather than a single substance since at least 6 crystalline steroids and an amorphous fraction, having the same biological properties as extracts of post-operative urine, have been isolated from the adrenal cortex.

Indirect evidence favoring the adrenal origin of these substances is provided by a consideration of the amounts present in urine under different circumstances. Thus the urine excreted by man following exposure to stress, such as burns, infections, and operative procedures, contains 3 to 30 times the amount of cortin-like substances (assayed by the cold exposure test) that is excreted by normal individuals. It has been demonstrated by Selye (1940) that under similar conditions

of stress the adrenal cortex of the rat loses its lipids and subsequently undergoes hypertrophy which may be taken as indirect evidence of increased secretory activity. The increased excretion of the cortin-like substances thus occurs at a time when it might be expected that there is an increased production of adrenal cortical hormones in man. Further evidence supporting the view that stress in man is accompanied by increased activity of the adrenal cortex is summarized by Albright (1943) in a recent review.

Little information is available regarding the metabolism of the adrenocortical steroids. That urinary neutral 17-ketosteroids may be derived from the adrenal cortex is indicated by the following facts: (a) similar 17-ketosteroids such as androstane-3 (β), 11 (β)-diol-17-one, Δ^4-androstene-3, 11, 17-trione and Δ^4-androstene-3, 17-dione (Reichstein and Shoppee (1943) have been isolated from extracts of the adrenal cortex, (b) eunuchs (Callow and Callow, 1940) and ovariectomized women (Hirschmann, 1941) excrete 17-ketosteroids in very nearly normal amounts, (c) some patients with neoplasm or hyperplasia of the adrenal cortex excrete a markedly increased amount of 17-ketosteroids (Talbot, Butler and Bergman, 1942) while (d) patients with Addison's disease excrete little or no 17-ketosteroids (Fraser, Forbes, Albright, Sulkowitch and Reifenstein, 1941). Similar evidence indicates that the urinary estrogens may in part be derived from the adrenal cortex. In addition a number of steroid alcohols, pregnane-3 (α), 17, 20-triol, pregnane-3 (β), 17, 20-triol (?) (Butler and Marrian, 1937, 1938 a, b), pregnane-3 (α), 20 (α)-diol (Venning, Weil and Browne, 1939) and Δ^5-androstene-3 (β), 16, 17, -triol (Hirschmann, 1943), have been isolated in considerable quantities from the urine of patients suffering from adrenocortical hyperplasia or carcinoma and their origin from the adrenal cortex is thus indicated.

Current views on the metabolic interrelationships of the adrenocortical steroids and the urinary steroids mentioned above are derived from correlation of the structural formula of the steroids from both sources. Only in the case of desoxycorticosterone has the metabolism of a biologically active adrenocortical steroid been studied in the classical manner. Following the administration of this compound, as the acetate, to man (Cuyler, Ashley and Hamblen, 1940), rabbit (Westphal, 1942; Hoffman, Kazmin and Browne, 1943) and to the chimpanzee (Fish, Horwitt and Dorfman, 1943) pregnanediol was isolated from the urine.

Few experiments concerned with the detection of biologically active substances in the urine following the administration of adrenal cortical steroids have been reported. Pfiffner, Swingle and Vars (1934) were unable to demonstrate cortical activity at a level of 200 dog units in the urine of a dog which had been injected subcutaneously with an adrenal cortical extract containing 2340 dog units. Harrop and Thorn (1937), on the other hand, recovered from the urine 10%

of the biological activity (effect on salt and water metabolism) of an adrenal cortical extract, containing 3000 dog units, administered intravenously to normal dogs. The experiments herein described indicate that when relatively large doses of adrenal cortical extract are administered intravenously to man, 7 to 12.3% of the activity of the extract, in terms of substances active in the cold exposure test, are recovered from the urine. Similarly Weil (1941) observed an increase in urinary substances active in the cold exposure test equivalent to 25% of the activity of an adrenal cortical extract (100 cc.) administered intravenously to a patient with Addison's disease. The failure of Pfiffner, Swingle and Vars to demonstrate activity in the urine of the dog treated with adrenal cortical extract may be explained by the fact that in their experiment the extract was given subcutaneously.

The present experiments and those of Harrop and Thorn have shown that a considerable proportion (approximately 10%) of the biologically active substances of an adrenal cortical extract administered intravenously to man and dog is excreted in the urine. Vogt (1943), however, concluded that renal excretion plays only a negligible role in the disposal of cortical hormone as she was unable to demonstrate any accumulation of this hormone in the general circulation of the eviscerated, nephrectomized cat.

SUMMARY

A method is described for the preparation of extracts of postoperative urine which have the capacity: (a) to maintain adrenalectomized rats exposed to cold, (b) to induce deposition of glycogen in the liver of the fasted adrenalectomized rat, and, (c) to sustain life and growth of the adrenalectomized rat. The process of purification affords on the average a 100 fold concentration of the cortin-like substances with a loss of approximately 25% of the biological activity present in the initial crude extracts. The final extract, which is ketonic, contains 25–100 cold units per mg. (100 cold units are equivalent to 1 mg. of crystalline corticosterone).

Individuals exposed to the stress of surgical procedures were found to excrete 3–30 times the amount of cortin-like substances that is excreted by normal men. This finding is interpreted as supporting the hypothesis that the urinary cortin-like substances may be metabolites of the adrenocortical hormones.

It has been shown that 7 to 12.3% of the biological activity (as measured by the cold exposure test) of adrenal cortical extract administered intravenously to man is recoverable from the urine.

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REFERENCES

ALBRIGHT, F.: The Harvey Lectures, Series 38: 123. 1942–43.
ANDERSON, E. M., AND W. HAYMAKER: *Science* 86: 545. 1937.
ANDERSON, E. M., AND W. HAYMAKER: *Proc. Soc. Exper. Biol. & Med.* 38: 610. 1938a.
ANDERSON, E. M., W. HAYMAKER AND M. JOSEPH: *Endocrinology* 23: 398. 1938b.
BUTLER, G. C., AND G. F. MARRIAN: *J. Biol. Chem.* 119: 565. 1937.
BUTLER, G. C., AND G. F. MARRIAN: *J. Biol. Chem.* 124: 237. 1938.
CALLOW, N. H., AND R.K. CALLOW: *Biochem. J.* 34: 276. 1940.
CUYLER, W. K., C. ASHLEY AND E. C. HAMBLEN: *Endocrinology* 27: 177. 1940.
DORFMAN, R. I., AND B. N. HORWITT: *Federation Proc.*, 2: 60. 1943.
DORFMAN, R. I., B. N. HORWITT AND W. R. FISH: *Science* 96: 496. 1942.
FISH, W. R., B. N. HORWITT AND R. I. DORFMAN: *Science* 97: 227. 1943.
FRASER, R. W., A. P. FORBES, F. ALBRIGHT, H. SULKOWITCH AND E. C. REIFENSTEIN: *J. Clin. Endocrinol.* 1: 234. 1941.
GOOD, C. A., H. KRAMER AND M. SOMOGYI: *J. Biol. Chem.* 100: 485. 1933.
GROLLMAN, A., AND W. M. FIROR: *Proc. Soc. Exper. Biol. & Med.* 30: 669, 1932–33.
GROLLMAN, A., AND W. M. FIROR: *J. Biol. Chem.* 100: 429. 1933.
HARROP, G. A., AND G. W. THRON: *J. Clin. Investigation* 16: 659. 1937.
HARTMAN, F. A.: *Endocrinology* 14: 229. 1930
HIRSCHMANN, H.: *J. Biol. Chem.* 136: 483. 1940.
HIRSCHMANN, H.: *J. Biol. Chem.* 150: 363. 1943.
HOFFMAN, M. M., V. E. KAZMIN AND J. S. L. BROWNE: *J. Biol. Chem.* 147: 259. 1943.
HORWITT, B. N., AND R. I. DORFMAN: *Science* 97: 337. 1943.
KENDALL, E. C.: *Arch. Path.* 32: 474. 1941.
PERLA, D., AND J. MARMORSTON-GOTTESMAN: *Proc. Soc. Exper. Biol. & Med.* 28: 1024. 1931.
PFIFFNER, J. J., W. W. SWINGLE AND H. M. VARS: *J. Biol. Chem.* 104: 701. 1934.
REICHSTEIN, T.: *Helv. Chim. Acta.* 19: 29. 1936.
REICHSTEIN, T., AND C. W. SHOPPEE: Vitamins and Hormones. 1: 346. 1943.
REINECKE, R. M., AND E. C. KENDALL: *Endocrinology* 31: 573. 1942.
ROOS, A.: *Endocrinology* 33: 276. 1943.
SCHILLER, S., AND R. I. DORFMAN: *Endocrinology* 33: 402. 1943.
SELYE, H.: *Cycl. Med. Surg. and Spec.* 15: 15. 1940.
SELYE, H., AND C. DOSNE: *Lancet* July 20: 70. 1940.
SELYE, H., AND V. SCHENKER: *Proc. Soc. Exper. Biol. & Med.* 39: 518. 1938.
SHIPLEY, R. A., R. I. DORFMAN AND B. N. HORWITT: *Am. J. Physiol.* 139: 742. 1943.
TALBOT, N. B., A. M. BUTLER AND R. A. BERMAN: *J. Clin. Investigation* 21: 559. 1942.
TYSLOWITZ, R., AND E. B. ASTWOOD: *Am. J. Physiol.* 136: 22. 1942.
VENNING, E. H., P. G. WEIL AND J. S. L. BROWNE: *J. Biol. Chem.* 128: cvii. 1939.
VENNING, E. H., M. M. HOFFMAN AND J. S. L. BROWNE: *J. Biol. Chem.* 148: 455. 1943.
VOGT, M.: *J. Physiol.* 102: 341. 1943.
WEIL, P. G., AND J. S. L. BROWNE: *Science* 90: 445. 1939a.
WEIL, P. G., AND J. S. L. BROWNE: *Am. J. Physiol.* 126: 652. 1939b.
WEIL, P. G., AND J. S. L. BROWNE: *J. Clin. Investigation* 19: 772. 1940.
WEIL, P. G., AND J. S. L. BROWNE: In press, 1944.
WEIL, P. G.: Adrenal Cortex and Its Role in Resistance, McGill University, Montreal, 1941.
WESTPHAL, U.: *Z. Physiol. Chem.* 273: 13. 1942.
ZARROW M.: *Proc. Soc. Exper. Biol. & Med.* 50: 135. 1942.

EVIDENCE FOR EARLY TESTIS HORMONE SECRETION IN THE RAT FROM A STUDY OF THE EPIDIDYMIS[1]

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THE EARLIEST PERIOD at which testis hormone secretion is first demonstrable in the rat has not been established. Incidental observations made on the colony of rats bred in this laboratory (Moore, 1930; Price, 1936) by using as an indicator the secretion granules in the secretory epithelium of seminal vesicles indicate that hormone secretion is evident by about the 35th day of post-natal life. Secretory cells of the ventral prostate show light areas in the cytoplasm at still earlier ages, and in the adult male these are adequate to demonstrate the presence of testis hormone. Price (1936), however, showed that these light areas develop in the prostate of a male, castrated on the first day of life, although the prostate was smaller than normal and the appearance of light areas was delayed to some extent (day 12 in normal; day 17 in castrates); she suggested these findings indicate an influence from adrenal androgens (Price, 1939; Howard, 1937; Burrill, 1939). It follows, therefore, that in the use of the prostate as a testis hormone indicator in early post-natal life histological criteria alone may be misleading in indicating a testicular secretion, but good evidence was presented by Price that testis hormone secretion was evident by the 15th day. Although Wiesner (1934–35) presented evidence from the rat that led him to suggest that testis hormone secretion was operative prior to birth, the idea scarcely appears to be satisfactorily demonstrated.

The early reactivity of gonads and accessory reproductive organs of the rat to hormones of various types has been studied by Price and Ortiz. It became of some interest to determine the capability of the epididymis to serve as an indicator organ for testis hormone in young post-natal rats. Towards this goal the caudal portion of the epididymis of 96 rats autopsied between the 6th and 45th post-natal day has been weighed carefully on a torsion balance in order to determine (1) whether, or at what age, the epididymis of castrated animals revealed

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consistent differences in weight from controls, and (2) the responsiveness of the organ to injection of androgens.

MATERIALS AND METHODS

Young rats were castrated under ether anaesthesia on the first day after birth in the majority of cases; a small number were not operated until day 2, 3 or 4. Castration was performed with the use of a wide field binocular microscope, through an incision just posterior to the genital tubercle; slight abdominal pressure brought the testis into clear view. A cut passing through the body of the epididymis permitted removal of the testis, and the retention of the tail of the epididymis and vas deferens which was to be employed as the test organ; the artery of the vas deferens supplies this portion of the epididymis. In attempting to determine the possible effects of castration on days 1 to 4 on the tail of the epididymis, comparisons were made between the weights of this organ from pairs of males from the same litter, autopsied on the same day, and in which the testes and head of each epididymis had been removed from one of the pair and an identical operation done on the second with the difference that the testes were not removed. In some cases either two pseudo-operated controls were compared with a single castrate, or two castrates compared with a single control in which case average weights of the two were used and the group considered as a pair. A given litter frequently provided pairs that were autopsied at different ages.

RESULTS

Effects of Castration on the Epididymis. The animals employed for a study of the caudal portion of the epididymis are listed in table 1 as 28 littermate pairs of males, one of each of the pairs having been castrated while the other served as control; the control in all pairs autopsied up to day 30 had been subjected to an identical operation with the exception that testes were not removed, and the control in all older cases of autopsy had not been subjected to operation. In six cases among controls and one case among the castrates the average weight of the caudal epididymis from two littermate males was used; in all cases epididymal weight is the combined weights of both epididymides from an animal.

An examination of table 1 will reveal that the weight of the epididymis of the castrated partner was lower than that of the littermate control in 27 of the 28 pairs listed; the single exception occurs in the pair autopsied on day 7. The response of the epididymis to castration is expressed for each pair as a percentage decline in the weight of the epididymis of the castrate when compared with the weight of the epididymis of the pseudo-operated or unoperated littermate control. The variability in weight reduction in the different pairs is great, but a general trend towards progressively greater differences with increased age can be seen when the averages of percentage declines for different periods are computed. Examination of animals autopsied during different ten day periods would show the following average

per cent decline for castrates, autopsies day 6–10, 9 per cent; days 11–20, 30 per cent; days 21–30, 25 per cent; days 31–40, 41 per cent; days 41–45, 62 per cent. A division into two age groups (days 6 to 20 and days 21 to 45) shows that the average percentage decline of castrates in the younger group is 21 per cent below that of their controls, whereas in the older group the average decline is 50 per cent.

A histological examination of the lower portion of the epididymis was made on five pairs autopsied on days 8, 13, 18, 28 and 41 in order

TABLE 1. EFFECTS OF CASTRATION ON WEIGHT OF TAIL OF EPIDIDYMIS IN RATS

Age, in days		Pseudo-operated, or normal control[1]		Castrated		Percentage decrease in in castrates
At operation	At autopsy	No. of animals	Weight of epididymis (mg.)	No. of animals	Weight of epididymis (mg.)	
1	6	1	3.8	1	3.2	15
1	7	2	4.5	1	5.4	20 (increase)
1	8	1	6.4	1	5.6	12
1	8	2	4.9	1	4.2	14
1	8	2	5.2	1	3.8	26
1	8	1	10.6	1	8.6	18
1	10	2	6.8	1	6.0	11
1	10	1	6.4	1	6.2	3
1	11	2	7.7	1	4.6	40
1	11	1	8.0	1	4.4	45
1	12	1	5.6	1	4.0	28
1	13	1	8.0	1	5.4	32
1	13	1	5.8	1	4.0	31
1	13	1	5.8	1	5.6	3
1	14	2	9.2	1	6.6	28
1	15	1	8.0	1	5.6	30
1	15	1	12.8	1	6.4	50
3	18	1	11.6	1	9.2	20
3	23	1	10.0	2	9.7	3
2	28	1	12.2	1	6.4	47
4	31	1	27.6	1	5.6	79
2	38	1	28.2	1	27.4	3
2	41	1	47.4	1	18.0	62
4	41	1	23.0	1	7.0	70
4	41	1	50.0	1	39.4	21
4	45	1	79.0	1	12.8	84
4	45	1	49.0	1	11.4	76
2	45	1	74.0	1	30.8	58

[1] Controls in all pairs autopsied up to 31 days of age were pseudo-operated (testes intact); controls in pairs autopsied days 31 to 45 were unoperated.

to find some character to serve as a basis for comparison. From these samples no good criterion could be established.

Responsiveness of the Epididymis to Injection of Androgens. Since the removal of the testes was followed by a consistent reduction in the weight of the epididymis of animals at very early ages, thus suggesting that the greater weight of the normal epididymis reflected the presence of hormones secreted by the intact testes, it became of interest to determine whether the injection of small amounts of androgens would lead to increases above the uninjected controls.

As in the previous group littermate males were employed and comparisons were made between the weights of the caudal epididymis of controls (pseudo-operated and castrated) each of which received daily injections of .01 cc. of sesame oil and castrates injected daily with small amounts of testosterone propionate. In table 2 it will be seen that the weight of the epididymis of the hormone treated castrates was greater in eleven of the thirteen paired comparisons, two of which contained two controls and two hormone treated castrated animals.

TABLE 2. EFFECTS OF ANDROGENS ON WEIGHT OF TAIL OF EPIDIDYMIS IN RATS[1]

Age in days at autopsy	Control		Injected castrates			Percentage increase in injected castrate
	No. of animals	Weight of epididymis (mg.)	No. of animals	Daily dosage T-prop. (mg.)	Weight of epididymis (mg.)	
		pseudo-oper.				
8	1	4.6	1	.01	3.6	21 (decrease)
8	1	4.6	1	.01	5.0	8
10	1	4.0	1	.01	4.8	20
10	1	6.0	1	.01	7.6	26
10	1	5.4	1	.01	5.6	3
10	1	5.8	1	.01	8.2	41
13	1	6.4	1	.01	4.2	34 (decrease)
13	1	5.2	2	.01	7.9	51
15	1	6.0	2	.01	14.6	143
		castrate				
10	2	5.5	2	.05	7.2	30
10	1	3.6	1	.01	5.2	44
13	2	3.9	2	.05	12.0	233
13	1	3.8	1	.01	11.8	210

[1] All animals operated on day 1; injections day 3 to autopsy.

DISCUSSION

Since testis hormone detection depends upon demonstrable changes in organs or tissues it was the purpose of this investigation to determine whether the epididymis would show sufficiently clear changes in structure or weight to provide an indicator for the presence of testis hormone in the very young rat. In the event this organ revealed definite responses attributable to hormone it was desired to utilize it to determine how early in the life of the normal rat the hormone secreting activity could be determined.

The histological changes in the epididymis at very early ages did not prove to be usable criteria. Responses in the weight of the organ provided a more satisfactory criterion of the presence of hormone, and this criterion has been relied upon chiefly to indicate secretory activity of the testes.

The data presented in table 1 reveal that at autopsy of pairs of rats on the eighth day of life the weight of the epididymis of a male

castrated on the day 1 was consistently lower than that in a littermate subjected to an identical operation, with the difference that the testes were not removed. The consistent response at the age of 8 days (four pairs) coupled with similar responses at all other ages up to day 45 suggests that the weight differences reflect differences in the hormonal condition of the various pairs. In the event the epididymis could be shown to respond to the injection of hormones at early ages and produce organs greater in weight than the controls it would provide even more convincing evidence that the effects of castration depended upon the absence of hormone, and thus that hormone secretion by the testes was demonstrable by the eighth day of life. The results obtained by injections of small daily doses of testosterone propionate (table 2) prior to autopsy at ages up to the 13th day of life provide substantiating evidence that the changes noted actually depend upon hormones. It follows, therefore, that the testes of normal male rats secrete detectable amounts of hormone by the eighth day following birth.

SUMMARY

Castration of male rats one day after birth and autopsied from day 8 to day 45 leads to consistent reduction in the weight of the tail of the epididymis in comparison with pseudo-operated or normal control littermates. Injections of .01 mg. testosterone propionate daily from day 3 to autopsy on days 8 to 13 result in detectable increases in weight of the epididymis. Weight changes in the epididymis reveal that hormone secretion by testes in normal rats can be detected by the eighth day of life.

REFERENCES

Burrill, M. W., and R. R. Greene: *Proc. Soc. Exper. Biol. & Med.* 40: 327. 1939.
Howard, Evelyn: *Am. J. Anat.* 62: 351. 1937.
Moore, C. R., W. Hughes and T. F. Gallagher: *Am. J. Anat.* 45: 109. 1930.
Price, D.: *Am. J. Anat.* 60: 79. 1936.
Price, D.: *Proc. Soc. Exper. Biol. & Med.* 41: 580. 1939.
Price, D., and E. Ortiz: *Endocrinology* 34: 215. 1944.
Wiesner, B. P.: *J. Obst. & Gynaec. Brit. Emp.* 41: 867, 42: 8. 1934–35.

NOTES AND COMMENTS

EFFECT OF ILLUMINATION ON THE EGG-EXTRUSION REACTION OF *XENOPUS LAEVIS* IN THE FROG TEST FOR PREGNANCY[1]

NUMEROUS INVESTIGATORS have demonstrated that the South African Clawed Frog (*Xenopus laevis*) always extrudes its ova at night during its mating period (Leslie, 1890; Bles, 1901; Landgrebe and Purser, 1941; Gasche, 1943 and Weisman and Coates, 1944a). In recent years, this egg-extrusion reaction, in response to injections of urine containing gonadotrophic hormones, has been used as a diagnostic test for pregnancy (Bellerby, 1934; Shapiro and Zwarenstein, 1934; Elkan, 1938; Weisman and Coates, 1943; 1944b).

We are in receipt of correspondence from a number of workers, experimenting with this test animal, complaining of occasional poor oviposition reactions. Upon close scrutiny of each individual problem it was learned that these workers invariably conducted their tests with the animals exposed to the direct glare of either sunlight or electric light.

It occurred to us that since the normal oviposition reaction of *Xenopus laevis* takes place at night, and under water, that solar or electrical glare may act as inhibitors. This thought seemed even more logical since our own successful reactions have been performed in a fairly darkened part of our laboratories, away from the direct exposure to light, or else the tests were started at late afternoon and the animals kept in a room devoid of light during the night.

We accordingly decided to set up two series of test animals and compare the results obtained from gonadotropinic stimulation upon animals exposed to illumination with those found in *Xenopus* kept in total darkness.

EXPERIMENTAL

Sixteen two-gallon size test tanks were set up with one *Xenopus* in each tank. The animals in tanks numbered 1–8 were injected with increasing doses of a known active gonadotrophic hormone. This group of animals was kept in a dark room devoid of all sources of illumination. The frogs in tanks 9–16 were injected in a similar fashion but were kept exposed to continuous illumination. The source of light was derived from two 15 watt fluorescent lamps with the glare directed by reflectors to the test tanks. Observation of the animals for egg-extrusion was made at four hour intervals.

RESULTS

As is shown in the accompanying table, the South African Frogs maintained in darkness reacted by oviposition quickly and efficiently. The egg-extrusion reactions of *Xenopus laevis* exposed to direct light were definitely inhibited.

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TABLE 1. EGG-EXTRUSION IN *Xenopus laevis* IN DARKNESS AND UNDER DIRECT ILLUMINATION

Tank No.	Dose of the gonadotropin	Reaction in total darkness (Group A)	Tank No.	Reaction when exposed to direct illumination (Group B)
1	100 rat units	Strong reaction* within 8–16 hr.	9	Negative after 24 hr.
2	100 r.u.	Strong reaction within 8–16 hr.	10	Negative after 24 hr.
3	150 r.u.	Strong reaction within 8–16 hr.	11	Minimal in from 16–20 hr.
4	150 r.u.	Strong reaction within 8–16 hr.	12	Negative after 24 hr.
5	200 r.u.	Strong reaction within 8–16 hr.	13	Minimal in from 12–16 hr.
6	200 r.u.	Strong reaction within 8–16 hr.	14	Minimal in from 16–20 hr.
7	250 r.u.	Strong reaction within 8–16 hr.	15	Strong reaction within 8–12 hr.
8	250 r.u.	Strong reaction within 8–16 hr.	16	Minimal in from 12–16 hr.

* A strong reaction is indicated by a deposition of hundreds of eggs. A minimal reaction indicates extrusion of only a few eggs.

SUMMARY

Two groups of South African Frogs (*Xenopus laevis*) were injected with gonadotrophic hormones and tested for the effect of illumination on the egg-laying response in the test for pregnancy. The frogs tested in total darkness reacted normally and promptly by egg-extrusion. Those exposed to direct fluorescent illumination responded poorly or not at all. It is suggested that when *Xenopus laevis* is used as a test animal for the diagnosis of pregnancy, the frogs be removed from all sources of illumination.

REFERENCES

BELLERBY, C. W.: *Nature, London* 133: 494. 1934.
BLES, E. J.: *Proc. Camb. Philo. Soc.* 11: 220. 1901.
ELKAN, E. R.: *Brit. M. J.* 2: 1253. 1938.
GASCHE, P.: *Rev. Suisse de Zool. Geneva* 50: 262. 1943.
LANDGREBE, F. W., AND G. L. PURSER: *Nature, London* 148: 115. 1941.
LESLIE, J. M.: *Proc. Zool. Soc. London* p. 69. 1890.
SHAPIRO, H. A., AND H. ZWARENSTEIN: *Nature, London* 133: 762. 1934.
WEISMAN, A. I., AND C. W. COATES: The South African Frog (*Xenopus Laevis*) in Pregnancy Diagnosis, N. Y. Biologic Research Foundation, New York, 1944a.
WEISMAN, A. I., AND C. W. COATES: *Bull. N. Y. Acad. Med.* 19: 660. 1943.
WEISMAN, A. I., AND C. W. COATES: *West. J. Surg.* 52: 171. 1944b.

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A STUDY ON INACTIVATION OF OVARIAN HORMONES BY THE LIVER

Many experiments have shown that the liver tissue of the dog, rat, mouse, rabbit, guinea-pig and frog (Silberstein, *et al.*, 1933; Zondek, 1934; Engel and Navratil, 1937; Heller, 1939 and 1940; Engel, 1941) rapidly inactivates estrogens.

It has also been shown that ovaries, transplanted into the mesentery (Golden and Sevringhaus, 1938), or pellets of estrogens implanted into the spleen (Biskind and Mark, 1939) lose their characteristic effect. The same result is observed when estrogens are injected into the spleen or into the portal circulation (Talbot, 1939; Glass, *et al.*, 1940; Biskind and Biskind, 1942; Biskind and Shelesnyak, 1942) and even after infrahepatic intravenous injection (Cantarow, Paschkis, Rakoff and Hansen, 1943). The assay of homo transplants of ovaries by Golden and Sevringhaus (1938) is especially interesting.

It was therefore of interest to study the influence of such ovarian transplantation into the mesentery, not only upon the vaginal cycle, but also upon the uterus since it was possible that the lack of effect of transplanted ovaries upon the uterus could be due either to the inactivation of estrogens or of progesterone. The inactivation of the latter by the liver has not been studied up to now. Therefore two series of experiments were made—(a) a study of the comparative influence of the transplantation of the ovaries into the portal and systemic circulation, and (b) to determine whether progesterone is destroyed by the liver.

Young adult female rabbits were used. From six animals one was kept as a control; the second spayed; in two animals the ovaries were transplanted into the mesentery; and in two animals the ovaries were transplanted into the large muscles of the back.

Six weeks after the operation, all the animals were killed. The transplanted ovaries were not atrophied. The uteri of the spayed animals showed a total atrophy of the glands, the mucous membrane and even the muscular layer. The uteri of the rabbits, whose ovaries had been transplanted into the mesentery, showed macroscopically as well as microscopically the same total atrophy as those of the castrate animals. The controls, and those in which the ovaries had been transplanted into the muscles of the back, showed no atrophy of the uterus. In a second series of six rabbits the same result was found.

These results show that in the rabbit ovarian hormones are inactivated by the liver and a complete atrophy of the uterus occurs after transplantation of the ovaries into an area drained by the portal circulation.

In order to test whether this atrophy was caused only by the inactivation of estrogens, or also by that of progesterone, the same technique which has been reported in a previous paper on estrogen inactivation was used (Silberstein, Engel and Molnar, 1933). Five mg. of progesterone were kept for twelve hours at 37° C. in contact with 100 gms. of liver pulp obtained from

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a freshly killed non-pregnant mare. The pulp was then extracted with alcohol and acetone and the extract suspended in olive oil. As control, the same quantity of progesterone was kept during the same time with fresh muscle pulp from the same horse.

As test animals infantile (600 gram) rabbits were used. The uteri were sensitized to progesterone by the daily injections of 0.005 mg. of diaethylstilbestrol (Lily) for eight days. This assay is based on the method of Clauberg (1933), who used infantile rabbits whose uteri were prepared with 20 mouse units of estrogen daily for eight days and then treated for five days with the corpus luteum extract.

The extract of the progesterone which had been in contact with the liver pulp was divided into three different portions, corresponding to three, two and one mgs. of progesterone. Three rabbits were treated with these extracts. The progesterone which had been exposed to the muscle pulp was treated in the same manner. Each portion of the extracts was divided into five equal doses and injected daily into the rabbits for five days. Then the animals were killed and the uteri examined.

In all the animals the characteristic progestational changes could be observed in the uteri. The mucosa was typically pregravid, enormously developed and there was no difference between the extracts treated with liver and those treated with muscle pulp.

It is therefore concluded that liver pulp does not inactivate progesterone. The atrophy of the uterus in animals whose ovaries had been transplanted into the portal circulation is exclusively caused by the inactivation of the ovarian estrogens. It is a remarkable fact that progesterone, which in its chemical structure is so similar to the other steroid sex hormones, is not inactivated by liver pulp, whilst stilbestrol with a very different chemical constitution is inactivated by the liver in vitro (Zondek, Sulman and Sklow, 1943).

SUMMARY

The uteri of rabbits whose ovaries had been transplanted into the mesentery show the same atrophy as those of spayed animals. Transplantation of the ovary into the large muscles of the back does not alter the ability of the ovarian estrogens to maintain a normal uterus.

Progesterone was not inactivated by liver pulp "in vitro."

REFERENCES

Biskind, M. S., and G. R. Biskind: *Endocrinology* 31: 109. 1942.
Biskind, G. R., and J. Mark: *Bull. Johns Hopkins Hosp.* 65: 212. 1939.
Biskind, M. S., and M. C. Shelesnyak: *Endocrinology* 30: 819. 1942.
Cantarow, A., K. E. Paschkis, A. E. Rakoff and L. P. Hansen: *Endocrinology* 33: 316. 1943.
Clauberg, C.: Die weiblichen Sexualhormone, Julius Springer, Berlin 1933.
Engel, P.: *Endocrinology* 29: 290. 1941.
Engel, P., and E. Navratil: *Biochem. Ztschr.* 292: 434. 1937.
Glass, S. J., H. A. Edmondson and S. N. Soll: *Endocrinology* 27: 749. 1940.
Golden, J. B., and E. L. Sevringhaus: *Proc. Soc. Exper. Biol. & Med.* 39: 361. 1938.
Heller, C. G.: *Endocrinology* 26: 619. 1940.
Heller, C. G., and E. J. Heller: *Endocrinology* 32: 64. 1943.

HELLER, C. G., E. J. HELLER AND E. L. SEVRINGHAUS: *Am. J. Physiol.* 126: 530. 1939.
SILBERSTEIN, F., P. ENGEL AND K. MOLNAR: *Klin. Wchnschr.* 12: 1693, 1933.
TALBOT, N. B.: *Endocrinology* 25: 601. 1939.
ZONDEK, B.: *Skandinav. Arch. f. Physiol.* 70: 133. 1934.
ZONDEK, B., F. SULMAN AND J. SKLOW: *Endocrinology* 33: 333. 1943.

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